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TITLE: Biomarkers of Oxidative Injury and Their Modulation in Prostate Tissue from Patients with Prostatic Tissue from Patients with Prostate Cancer

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13. ABSTRACT ***(Maximum 200 Words)***

The development of potent prevention strategies to diminish the threat of prostate cancer (PCa) are in order and remains the long-term goal of this project. One possible etiologic factor in the development of PCa, is cellular exposure to chronic oxidative stress. Chronic oxidative damage can lead to the accumulation of potentially promutagenic oxidized DNA bases such as 8-hydroxydeoxyguanosine (8-OHdG). The detoxifying enzyme *GSTP1* is inactivated in nearly 100% of PCa and is also frequently inactivated in prostatic intraepithelial neoplasia lesions. We have successfully developed a model system to determine the role of GSTP1 protein in the response of human PCa to chronic oxidative stress. We exposed the human PCa cell line, LNCaP (no GSTP1 protein expression) and subclones of LNCaP engineered to overexpress GSTP1, *in vitro*, to chronic oxidative stress inflicted by protracted, low dose irradiation (LDR) and arsenic trioxide. These experiments reveal: a) LNCaP exhibits an improved survival following exposure to LDR and arsenic trioxide compared to the GSTP1-overexpressing subclones and b) LNCaP accumulates greater amounts of 8-OHdG following this oxidative damage than the GSTP1-overexpressing subclones. Together, these data solidify our preliminary results and implicate *GSTP1* as a major regulator of oxidative DNA damage and suggest that its inactivation may provide a necessary step in the neoplastic process in prostate cancer. Importantly, we have recently learned that measurement of 8-OHdG and other oxidized DNA base adducts is more accurately performed by HPLC-MS-MS than by HPLC-ECD, a method we typically employ. Given this reality, we have purchased, accepted and calibrated a new HPLC-MS-MS (PE Biosystems) and developed a rigorous method for determination of 8-OHdG in DNA from biologic samples. These new methods will allow for a more rapid and quantitative means of determining oxidized DNA base adduct levels from our LNCaP xenograft studies (nearly completed). More importantly, these new methods will also be in place before we begin to receive human prostatic tissue from men undergoing radical retropubic prostatectomy in our proposed chemoprevention trial.

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Introduction

Reactive oxygen species (ROS) inflict damage on many cellular components, including genomic DNA. Genome lesions accompanying ROS exposure include oxidized bases (1) and have been proposed to be promutagenic in certain systems (2, 3). We hypothesize that prostate epithelial cells with common genetic alterations are provided a significant survival advantage by these alterations when faced with an otherwise toxic oxidative stress. Inactivation of the pi-class glutathione S-transferase gene, *GSTP1*, most commonly occurring by promoter hypermethylation, appears to uniformly accompany human prostatic carcinogenesis (4) and most prostatic intraepithelial neoplasia (PIN) lesions (5). Glutathione S-transferases have been postulated to participate in the defense of normal cells against a variety of carcinogens by catalyzing conjugation reactions between reduced glutathione and reactive electrophiles and oxidants (6). This leads to the possibility that a deficiency in inducible GSTP1 enzyme activity in prostatic epithelial cells might substantially limit their electrophile and oxidant defense capabilities. Genetic alterations that do result as a consequence of oxidative damage are particularly important as they prevent the cell from activating cell death pathways in the face of DNA damage thereby allowing the cell to accumulate potentially promutagenic oxidized DNA adducts like 8-OHdG. Therefore, we reasoned that PCa cells devoid of GSTP1 might be tolerant to chronic oxidative stress and accumulate promutagenic DNA adducts, thereby increasing the risk of neoplastic transformation. Fortunately, there are a number of pharmacologic agents that have anti-oxidant properties and a number of others which specifically target enzymes known to be involved in the production of ROS. There is recent data suggesting several of these agents, including vitamin E and sulindac, may be protective against prostate and other epithelial cancers (7) (8). In fact, there is *in vitro* data suggesting that human PCa cell line growth is also inhibited by sulindac treatment (9). There is conflicting data on whether consumption of any of these agents can produce a decrement in the type and number of promutagenic DNA adducts (10, 11).

The hypotheses of this study are that **(a)** accumulation of oxidized DNA adducts following the chronic oxidant stress of LDR of human prostate cancer cells grown *in vitro* and *in vivo* is a result of *GSTP1* gene inactivation, **(b)** prostatic cells from patients with PCa will possess elevated amounts of oxidized DNA adducts as a result of the chronic oxidant stresses to which they have been exposed, and **(c)** the accumulation of these potentially harmful oxidative DNA adducts can be modulated by the treatment of patients with particular pharmacologic agents. In order to determine if our hypotheses are correct, the following studies are underway: (1) Expand upon and evaluate assays of oxidative DNA damage in the *in vitro/in vivo* LNCaP and LNCaP GSTP1 subclone human PCa model we have developed. (2) Determine the amount of 8-OHdG and other markers of oxidative damage in the DNA of prostatic tissue samples from patients undergoing radical retropubic prostatectomy (RRP) for adenocarcinoma of the prostate. (3) Assay for modulation of 8-OHdG levels in the DNA of prostatic tissue samples from patients following treatment with new and developing pharmacologic prevention/anti-oxidant strategies. Together, these experiments will provide some of the first data set on the role of oxidative DNA damage and its modulation in human prostate cancer and assist in the development of rational PCa chemoprevention strategies.

Body

Task 1: Determine the amount of oxidized DNA base accumulation in our model system of human PCa cells with defective GSTP1 genes as well as those engineered to express GSTP1 polypeptide with enzyme activity following chronic oxidative stress delivered by protracted, low dose irradiation in vitro. Male nude mice will be injected with LNCaP and LNCaP-sublines.

We have completed our *in vitro* analysis of oxidative damage-induced cell death and oxidized DNA base damage accumulation in the human prostate cancer cell line LNCaP, the neo-control line and the three LNCaP-GSTP1 expressing sublines. These data reveal that when compared to any of the GSTP1-expressing sublines, LNCaP and the LNCaP-neo control cell line, both possessing inactivated *GSTP1* alleles, exhibit significantly greater survival following exposure to the cytotoxic effects of oxidative induced injury inflicted by protracted, low dose radiation (**Figure 1A**) as well as treatment with arsenic trioxide (**Figure 1B**).

Figure 1A

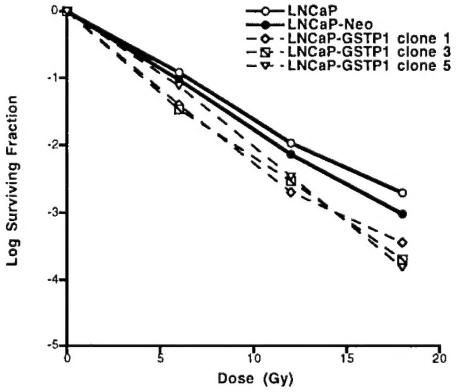
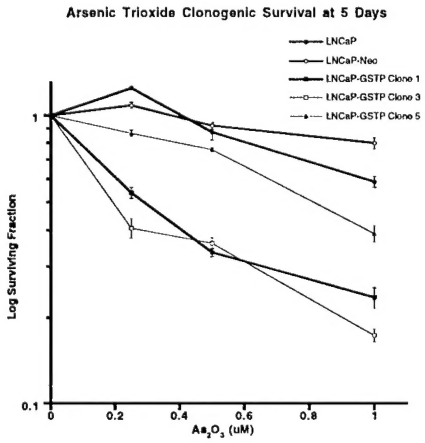


Figure 1B



Our previous work using G.C.-mass spectrometry revealed a significant accumulation of oxidized DNA base adducts, including 8-OHd-guanine, following radiation exposure in the LNCaP neo-control line when compared to the LNCaP-GST-expressing lines. Many investigators believe that HPLC-mass spectrometry (electrospray) (LC/MS-MS) provides a more accurate determination of oxidized DNA base adduct levels and one less likely to produce artifactual oxidation of the DNA during processing. As quantitation of 8-OHd-guanine in DNA samples is critical to the aims of this project, LC/MS-MS has an apparent advantage, particularly when measuring levels from human tissue, where basal levels of oxidized DNA adducts are thought to be low. To this end, we have acquired a new LC/MS-MS (P.E. Biosystems), developed new techniques for the measurement of oxidized base adducts by LC/MS-MS from biologic samples and have shown our ability to measure 8-OHd-guanine at femtomolar (fmol) concentrations. These experiments confirm that LC/MS-MS can accurately and reproducibly measure 8-OHd-guanine in our hands and also suggest that LC/MS-MS is better able to detect low levels (fmol) of oxidized DNA base adducts, which is superior to our previous G.C.-MS technique, (see **Appendices** for example). We have repeated

several *in vitro* protracted, low dose radiation exposure experiments of LNCaP and its GST-expressing clones and measured oxidized base adduct accumulation confirming our previous data set. Moreover, these data now allow us to confidently apply LC/MS-MS in the determination of oxidized DNA base adduct accumulation in prostatic tissue derived from our clinical trial which seeks to determine the ability of candidate chemopreventive agents to modulate these potentially promutagenic adducts.

Task 2: Ascertain levels of oxidized DNA base accumulation in LNCaP and GSTP1-subclone xenografts grown in male nude mice. Simultaneously perform immunohistochemical staining for PCNA and p27 polypeptides. Correlation of these data with those from the high performance liquid chromatography-electrochemical detection (HPLC-ECD) and gas chromatography/mass spectrometry with selected ion monitoring (GC/MS-SIM) analyses will be performed in an attempt to determine if PCNA and/or p27 may serve as potential "markers" of oxidative stress. IRB approval for Aim #2 studies will be obtained.

As detailed in **Task 1**, it became evident that a new detection method for oxidized DNA base adducts, LC/MS-MS, was superior to our HPLC-ECD method, thus requiring acquisition of new equipment and development of new sample preparation protocols. This has now been accomplished, allowing us to begin to analyze oxidized DNA base adducts in LNCaP and LNCaP-GSTP1 subclone xenografts. As of this report, we have no significant new measurements to share, but it is anticipated that these measurements will be completed in approximately 3 months.

Task 3: Identify and consent patients with adenocarcinoma of the prostate on whom a radical retropubic prostatectomy will be performed. Collect and prepare prostate tissue specimens and perform HPLC-ECD and GC/MS-SIM analyses. Tissue will also be prepared for immunohistochemistry. IRB approval will be obtained for Aim #3 clinical trial.

The protocol for the study of oxidized DNA base damage and immunohistochemical analysis of other potential markers of oxidative stress in radical prostatectomy specimens has been written, submitted to the Johns Hopkins Oncology Center's Clinical Research Committee for approval (**approved**) and submitted to the Johns Hopkins Institutional Review Board for approval (**approved**). The protocol, consent forms and approval letter are attached in the **Appendices**. Accrual of patients has commenced.

Task 4: Identify, consent and treat patients with adenocarcinoma of the prostate on whom a radical retropubic prostatectomy will be performed. These patients will be treated for 6 weeks with either one of two dose levels of vitamin E or a single dose level of sulindac. Collect and prepare prostate tissue specimens and perform HPLC-ECD and GC/MS-SIM analyses. Tissue will also be prepared for immunohistochemistry. Comparison of these data will be made with that from Task 3. Importantly, the Department of Urology at Johns Hopkins performs nearly 900 prostatectomies per year and nearly all patients will be eligible. If one were to consider only 20% of patients agree to study entry with a dropout rate of 25%, we

would easily be able to accrue 135 patients in one year, far in excess of the patients required for this non-randomized pilot study.

See Task 3 for details.

Key Research Accomplishments

- Development of a useful *in vitro* model of GSTP1 function in human prostate cancer based on the LNCaP cell line.
- Determination of the role of GSTP1 protein expression in the survival of human prostate cancer cells to the oxidative stress inflicted by low dose radiation as well as by arsenic trioxide. Human prostate cancer cells with inactivated *GSTP1* alleles and no GSTP1 protein expression can survive these oxidative stresses significantly better than the same cells engineered to express high levels of GSTP1 protein.
- Development of assay techniques for the measurement of several oxidized DNA bases by LC/MS-MS from biologic specimens.
- Evaluation of oxidized DNA base adduct accumulation following oxidative stress inflicted by protracted, low dose radiation in human prostate cancer cells with and without GSTP1 protein. These experiments suggest that cells with inactivated *GSTP1* alleles and no GSTP1 protein expression not only survive the oxidative stress of protracted, low dose radiation, but also accumulate significantly greater amounts of the potentially promutagenic oxidized bases, 8-OHd-guanine and 8-OHd-adenine.

Reportable Outcomes

Development of relevant human prostate cancer cell lines with and without GSTP1 protein expression.

Conclusions

Taken together, these data suggest that the most common genetic alteration known in human prostate cancer, hypermethylation of the *GSTP1* promoter leading to gene inactivation, can result in: 1) oxidative DNA damage tolerance as reflected by an increased clonogenic survival following protracted low dose radiation and exposure to arsenic trioxide, and 2) accumulation of potentially promutagenic oxidized DNA base adducts. These critical alterations in phenotype based on GSTP1 expression may be some of the earliest and most critical factors in the neoplastic process of human prostate cancer. These observations also solidify the concept of chemoprevention as a method of minimizing cellular oxidative DNA damage and provide a rationale for the choice of candidate chemopreventive agents in this study, vitamin E and the non-selective COX-inhibitor, sulindac.

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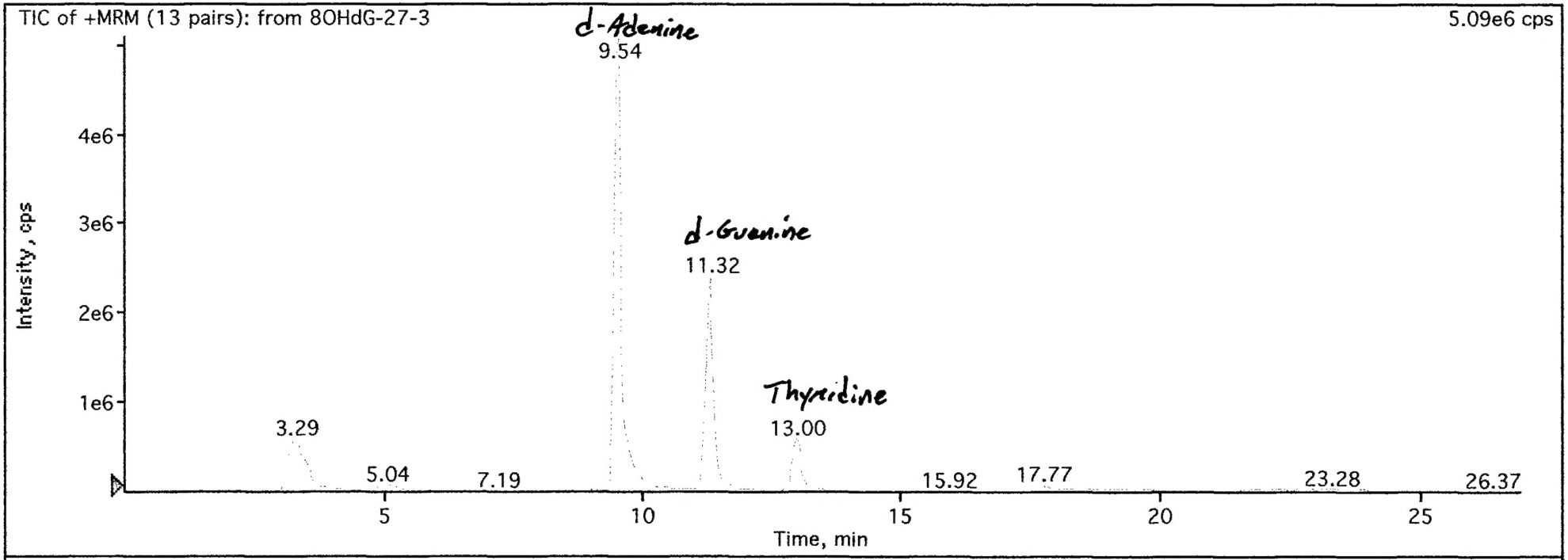

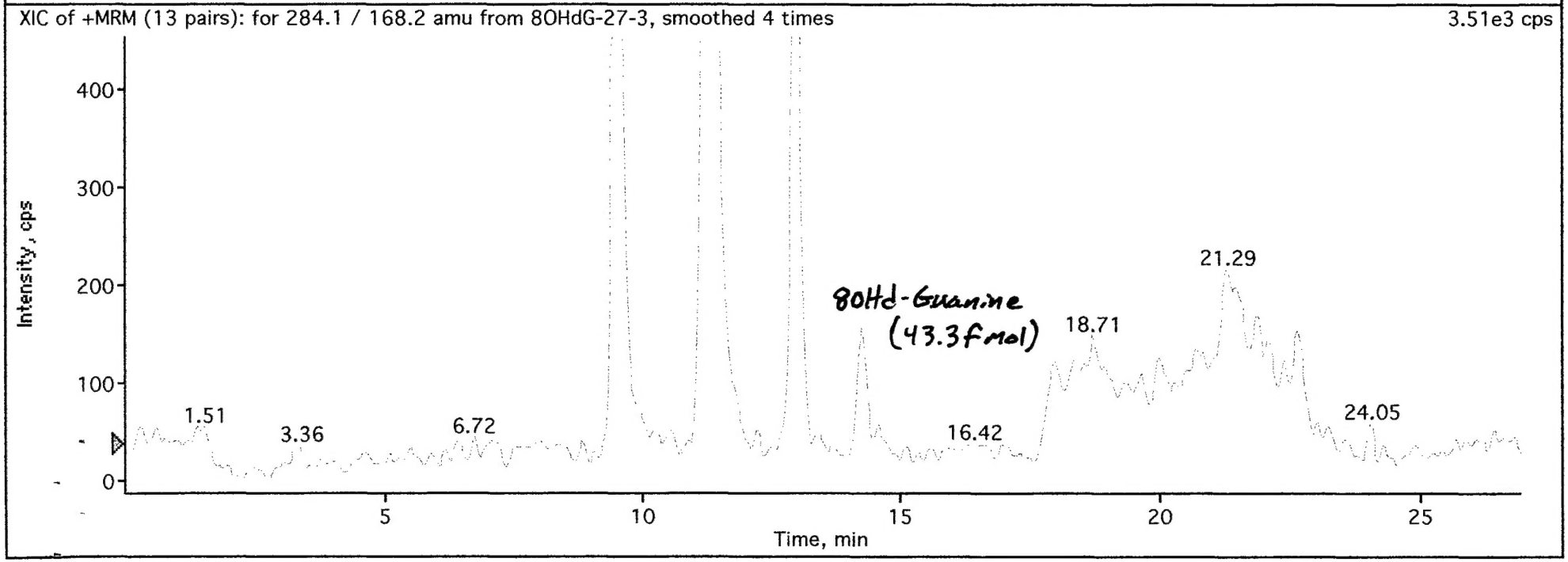

The Johns Hopkins University School of Medicine
The Johns Hopkins Hospital

Joint Committee on Clinical Investigation

JCCI PROTOCOL APPROVAL NOTICE

Please address reply to:
Turner 36 / School of Medicine
720 Rutland Avenue / Baltimore MD 21205-2196

(410) 955-3008
FAX (410) 955-4367

TO: Theodore L. DeWeese, MD
Assistant Professor, Oncology

FROM: Thomas R. Hendrix, M.D.
Chairman - JCCI

DATE: **February 13, 2001**

RE: **RPN NO.: 00-11-15-01, entitled, J0064 - A Pilot Study of Vitamin E and Sulindac in Men Pre-Prostatectomy for Clinically Localized Adenocarcinoma of the Prostate: Evaluation of Drug Specific Biomarker Modulation (with William Nelson, Michael Carducci, Angelo DeMarzo, Alan Partin, John Groopman) WITH REVISED CONSENT**

I am pleased to inform you that at the convened meeting of **02/13/2001** the JCCI voted to approve the above-referenced protocol. Approval of the protocol and the consent form(s) is for the period of **02/13/2001 to 02/13/2002.** As principal investigator of the project, you are responsible for fulfilling the following requirements of approval:

1) The co-investigators listed on the application should be kept informed of the status of the project.

2) Changes, amendments, and addenda to the protocol or the consent form must be submitted to the JCCI for re-review and approval prior to the activation of the changes. The RPN number assigned to the project should be cited in any correspondence.

3) Adverse events should be reported to the JCCI promptly. New information that becomes available which could change the risk:benefit ratio must be submitted promptly for JCCI review. The JCCI and outside agencies must review the information to determine if the protocol should be modified, discontinued, or continued as originally approved.

4) Only consent forms with a valid approval stamp may be presented to subjects. All consent forms signed by subjects enrolled in the study should be retained on file. The JCCI conducts periodic audits of protocol records, and consent documentation is part of such audits.

5) Federal regulations require review of an approved study not less than once per 12-month period. **Therefore, a renewal application must be submitted to the JCCI office SIX WEEKS prior to the above expiration date of 02/13/2002. This will allow sufficient time for review of the renewal application to be completed prior to the anniversary of the original approval date.** Failure to submit a renewal application in a timely fashion will result in termination of the study, at which point new subjects may not be enrolled and currently enrolled subjects must be taken off of the study.

CC: P&T, JHCC CRC
Enclosure

CLINICAL INVESTIGATION CONSENT FORM

The Johns Hopkins Medical Institutions
(The Johns Hopkins Hospital
The Johns Hopkins Bayview Medical Center, etc.)
Date/Revision: January 9, 2001 Application No: 00-11-15-01
Title of Research Project: A Pilot Study Of Vitamin E And Sulindac In Men Pre-Prostatectomy For Clinically Localized Adenocarcinoma Of The Prostate: Evaluation Of Drug-Specific Biomarker Modulation (J0064)

Patient I.D. Plate

Explanation of Research Project to Subject

PURPOSE OF STUDY

You have prostate cancer that appears to be limited to your prostate gland. You have opted to undergo surgery to have your prostate gland removed. It is expected that your surgery will be scheduled in the next 6 - 8 weeks. You are being asked to take part in a clinical trial which will compare individuals who receive study drugs prior to surgery versus individuals who receive "standard of care", that is, no therapy between the decision for surgery and actually having the operation. The study drugs are in pill form and could be used in the future to prevent prostate cancer. We are looking for changes in your prostate cancer after treatment with one of these drugs to determine if a much larger study makes sense to answer the question of whether these drugs may be helpful. Your participation in this study is voluntary.

The study drugs include a vitamin, Vitamin E and a drug used in severe arthritis called Sulindac. Studies suggest that these drugs may slow the progression of some cancers down or even prevent them from forming. You have cancer and we do not know whether these drugs can produce these effects in humans. Usually no special drugs are given before surgery, so if you agree to participate, you may be assigned to one of the study drugs or you may be assigned to no drugs. If you are assigned one of the drugs you will take your assigned drug for at least 6 weeks and up to 8 weeks prior to your surgery.

Nearly 60 patients will be enrolled in this study to take place at The Johns Hopkins Hospital. The study is sponsored by the United States Department of Defense.

PROCEDURES:

It will first be determined that you intend to undergo prostatectomy for the treatment of your prostate cancer. You will undergo a history and physical examination, and be asked to provide a list of all medications, including vitamins and herbs that you may take. Blood and urine samples will be obtained to assure your safety prior to starting the study drug.

You will be assigned Vitamin E, Sulindac, Vitamin E and Sulindac, or no study drug. Assignment will be in sequence. The first patient will receive Vitamin E, the second patient will receive Sulindac. The third patient will receive Vitamin E and Sulindac. The fourth patient will receive no study drug. The next four patients will receive the same sequence as the first four patients and all successive groups of four patients will continue in the same sequence until the study is complete. Both study drugs will come in pill form. If you are assigned Vitamin E, you will take the pills once per day. If you are assigned to Sulindac, you will take the pills twice a day. If you are assigned to both Vitamin E and Sulindac, you will take Vitamin E once a day and Sulindac twice a day. We ask you to save and return any pills that you do not use. If you take all the pills, we ask that you return the empty container. You will take the pills for at least six weeks before your surgery, but no more than 8 weeks. You will take the last dose of the medication on the night before your surgery.

THIS CONSENT FORM CONTINUES ON THE NEXT PAGE

You will be given a pill calendar and asked to mark off each day that you take the study drug. You will also be asked to list any side effects you may have to the drugs and to list any additional medications you are taking.

During the study you will be asked to return to the clinic to be evaluated for side effects and to follow-up on your blood tests. All other procedures are typical for a patient preparing for surgery at this institution. The blood tests may be done more often than is usually done in other patients similar to yourself. We will draw up to four tablespoons of blood with each sampling.

Your surgery will go on as planned. Your prostate gland will be removed and the pathologists will handle the tissue as they normally do. However, in your case, some portions of your tumor will be evaluated in research labs throughout the institution to examine if the study drug had any positive or negative effects. We will directly compare the results of those patients who received the study drug and those who did not.

You will not stay on the study drug after your surgery. You will be seen at your after surgery clinic visit to see if there were any other side effects noted by you.

We ask that you agree that some of the tissue from your surgery and some blood samples can be stored for further research. Your identity will remain confidential on any blood or tissue sample that is used for research. The investigators may look at your records, but your name will be kept private. If any of the results are published, once again you will not be identified.

RISKS AND DISCOMFORTS:

With any drug, there may be side-effects. Vitamin E is generally very well-tolerated. Rarely, it can cause nausea, diarrhea and intestinal cramps in some patients.

Overall, Sulindac is well-tolerated. It is somewhat similar to drugs like aspirin or ibuprofen. Side effects may include irritation of the stomach lining, nausea, vomiting, an increase risk for bleeding, and a possibility that the drug may slow your kidney function down. There may be other side effects which are not known. You will be followed closely and be seen by one of the doctors or nurses working on the study every other week.

The blood tests may cause minor pain where the needle is placed to remove blood. Besides pain, there may be bleeding, soreness, and the possibility of infection. These risks are of the procedure, not because your blood is being drawn more often.

There is the possibility that you may have a slight increase in bleeding during your surgical procedure. Your surgeons are aware of this risk and will take standard caution during the procedure to limit blood loss.

You may stop treatment at any time if you have side-effects or no longer wish to participate. If you wish to stop treatment, please let your doctor or the research nurse know of your decision. Your decision in no way will interfere with your planned surgical procedure. You will continue to receive care from your surgeon.

Costs to your or your insurance company for participating in this study are those costs which are considered standard for someone with your condition. The study drugs will be provided free of charge. The research studies and assays on your prostate tissue will not be charged to you. You will be asked to visit the research nurse during the six-eight weeks that you are on the study, and ;you will not be billed for those visits, unless it is your standard pre-operative visit to be scheduled by your doctor. As coverage by insurance carriers varies, you may wish to review your policy if questions regarding costs of clinical research are of concern.

THIS CONSENT FORM CONTINUES ON THE NEXT PAGE

If emergency treatment is needed as a result of this therapy or related to your cancer, you will be provided the best care possible at this institution. No money is available to reimburse you if emergency therapy is required.

BENEFITS:

There is no known benefit to participating in this study. Although these drugs may have some effects on your cancer cells, you may not receive it long enough to see improvement. We are looking for changes at a molecular level that will allow us to offer advice as to whether these drugs should be used more often. It is unlikely we will share with you the results of the molecular tests that we complete on your prostate tissue. Many of the tests are experimental and the meaning of their results is unclear at this time. We will not use any results to make decisions regarding your future care.

ALTERNATIVES TO PARTICIPATION:

Alternatives to participation in this study include no treatment at all until your surgery or other investigational drugs. You will receive the best possible care at this institution no matter what your decision.

You should understand that this study involves research. Rules are made during the planning stages of this study and are used to make sure that patients who enter this study are suitable and fit for this therapy and that they have the defined medical problem to be treated. For your own well-being, as well as to ensure that the results of this study can be useful for making treatment decisions regarding other patients with similar disease, it is important that no exceptions be made to these rules for admission to the study. You will receive a copy of this consent form.

THIS CONSENT FORM CONTINUES ON THE NEXT PAGE

QUESTIONS YOU MAY HAVE ABOUT THE RESEARCH STUDY:

This consent form explains the research study. Please read it carefully. Ask questions about anything you do not understand. If you do not have questions now, you may ask later. During the study, you will be told any new facts that could affect whether you want to stay in the study. If the study relates to a health problem you have, we will explain what other treatment could be given outside the research. You should understand those options before you sign this form. If you have questions you should call the principal investigator **Theodore L. DeWeese, M.D., at 410-955-7068.**

PRIVACY INFORMATION:

We will keep the study information private to the extent possible by law. However, State law requires us to report certain contagious diseases or if we find information about child abuse. Also, under certain conditions, people responsible for making sure that the research is done properly may review your study records. This might include people from Johns Hopkins, the National Institutes of Health, the Food and Drug Administration, or the sponsoring company (if any). All of these people are also required to keep your identity confidential. Otherwise, the information that identifies you will not be given out to people who are not working on the study, unless you give permission.

IN CASE OF INJURY:

If you are injured as a result of being in the study, or think you have not been treated fairly, please contact Dr. _____ at _____ (phone number). The services at the Johns Hopkins Hospital or the Johns Hopkins Bayview Medical Center will be open to you in case of any such injury. However, the Johns Hopkins University, the Johns Hopkins Hospital, the Johns Hopkins Bayview Medical Center, _____ and the Federal Government do not have a program to pay you if you are hurt or have other bad results which are not the fault of the study doctors.

You and your insurance company will be responsible for payment of any treatment or hospitalization you require if you are injured as a result of being in the study. It is up to you to check with your insurance company before you start this study to find out what your insurance company would pay for.

QUESTIONS ABOUT YOUR RIGHTS AS A RESEARCH SUBJECT:

If you have any questions about your rights as a subject in a research project, you should call the Joint Committee on Clinical Investigation at (410) 955-3008, or the Johns Hopkins Bayview Medical Center Institutional Review Board for Human Research (410) 550-1853 to receive help or advice.

JOINING OF YOUR OWN FREE WILL (Volunteering for the study):

You do not have to join this or any research study. If you do join, and later change your mind, you may quit at any time. If you refuse to join the study, you will not be penalized or lose any benefits to which you are otherwise entitled.

THIS CONSENT FORM CONTINUES ON THE NEXT PAGE

WHAT YOUR SIGNATURE MEANS:

Your signature below means that you understand the information given to you about the study and in this consent form. If you sign the form it means that you agree to join the study.

WE WILL GIVE YOU A COPY OF THIS CONSENT FORM.

STUDY APPROVED FOR ENROLLMENT OF: _x_Adults Only ___Adults and Children ___Children only

NOT VALID WITHOUT THE COMMITTEE OR IRB STAMP OF CERTIFICATION

APPROVED

FEB 13 2001

JOINT COMMITTEE ON CLINICAL INVESTIGATION

VOID ONE YEAR FROM ABOVE DATE
RPN NO 00-11-15-01
Form C (Revised 01/2001)

_____ Subject's signature (including children, when applicable) Date

_____ Signature of Parent or Legal Guardian (when applicable) Date

_____ Surrogate Signature for Subjects not Competent to Give Consent Date

Relationship of Surrogate to Subject: _____

_____ **Signature of Investigator or IRB/JCCI Approved Designee** Date

_____ Witness to Consent Procedures(Optional unless subject is illiterate, or unable to sign) Date

NOTE: A COPY OF THE SIGNED CONSENT FORM MUST BE KEPT BY THE PRINCIPAL INVESTIGATOR AND A COPY OF THE CONSENT FORM MUST BE PLACED IN THE PATIENT'S RECORD

Document Status:

Study Status:

Title: A Pilot Study of Vitamin E and Sulindac in Men Pre-Prostatectomy for Clinically Localized Adenocarcinoma of the Prostate: Evaluation of Drug-Specific Biomarker Modulation

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Action Dates: 1. JCCI Approval: 08/16/2000 (Submitted)
(Approved)

Keywords: DeWeese PI, Solid Tumor, Prostate, Vitamin E, Sulindac, Adenocarcinoma

PROTOCOL SYNOPSIS

TITLE OF PROTOCOL: A Pilot Study of Vitamin E and Sulindac in Men Pre-Prostatectomy for Clinically Localized Adenocarcinoma of the Prostate: Evaluation of Drug-specific Biomarker Modulation
PROTOCOL IRB NUMBER: Pending
PRINCIPAL INVESTIGATORS: Theodore L. DeWeese, M.D.
INSTITUTION: The Johns Hopkins Oncology Center, Bunting-Blaustein Research Building, 1650 Orleans St., Room 144, Baltimore, MD 21287
CO-INVESTIGATORS: William G. Nelson, M.D., Ph.D., Michael A. Carducci, M.D., Angelo DeMarzo, M.D., Ph.D., Alan W. Partin, M.D.,Ph.D., John Groopman, Ph.D.
STUDY CENTER: Johns Hopkins Oncology Center, The Brady Urological Institute, The Johns Hopkins University
STUDY AGENT: Vitamin E **FORMULATION**: Capsule **DOSE:** 200 I.U. **REGIMEN:** QD **ROUTE:** oral **STUDY AGENT:** Sulindac **FORMULATION**: Pill **DOSE:** 150 mg **REGIMEN:** BID **ROUTE:** oral
STUDY DESIGN: This single center trial will be a joint clinical project between the Johns Hopkins Oncology Center and the Johns Hopkins Brady Urological Institute. Laboratory measurement of endpoints will be conducted Johns Hopkins University. Eligible patients will receive either Vitamin at E 200 I.U. PO QD, Sulindac 150 mg PO BID, Vitamin E 200 I.U. PO QD and Sulindac 150 mg PO BID, or neither Vitamin E nor Sulindac (control group) for 6-8 weeks pre-prostatectomy. The assignment of treatment will be consecutive.
METHODOLOGY: The primary endpoint of this trial is to compare and correlate histologic and intermediate endpoints performed on prostate tissue from comparable groups of patients treated with Vitamin E and Sulindac for at least six weeks prior to prostatectomy with the same endpoints derived from previously collected untreated patients. Outcomes include histologic comparison, measurement of apoptosis and proliferation assays on treated/untreated tissue specimens, measurement of oxidative bases within treated/untreated tissue. Tertiary outcomes include patient compliance data.
STUDY DURATION: Phase I: Protocol preparation and review with necessary IRB approval (6 months) Phase II: Conduct of trial/ Patient enrollment (9 months), Laboratory Evaluation (Concurrent with patient enrollment = 9 months), Complete data analysis and final report (6 months) TOTAL PROJECT – 15 months.
PROPOSED ENTRY DATE OF FIRST SUBJECT: January 1, 2001
ESTIMATED COMPLETION DATE OF DOSING: September 30, 2001
PROPOSED ACCRUAL RATE : 5-6 subjects per month
DURATION OF TREATMENT: 6-8 weeks
FOLLOW UP: Patients will be screened/enrolled in the outpatient urology clinic of the Johns Hopkins Hospital. Initial staging, pathology review will take place prior to initiation of study drug. While on treatment, patients will have blood samples for PSA, free/total PSA, a serum bank pre-treatment, and every two weeks until surgery, and the day of surgery. At surgical removal, the specimen will be carefully handled and tissue distributed to the appropriate laboratories and collaborators as required.

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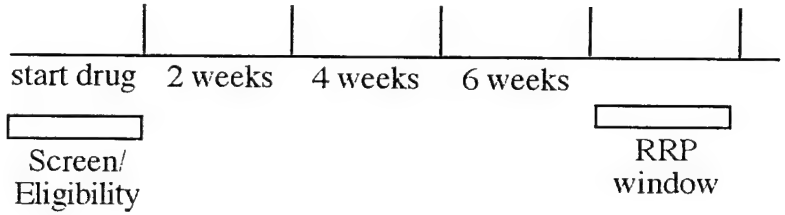
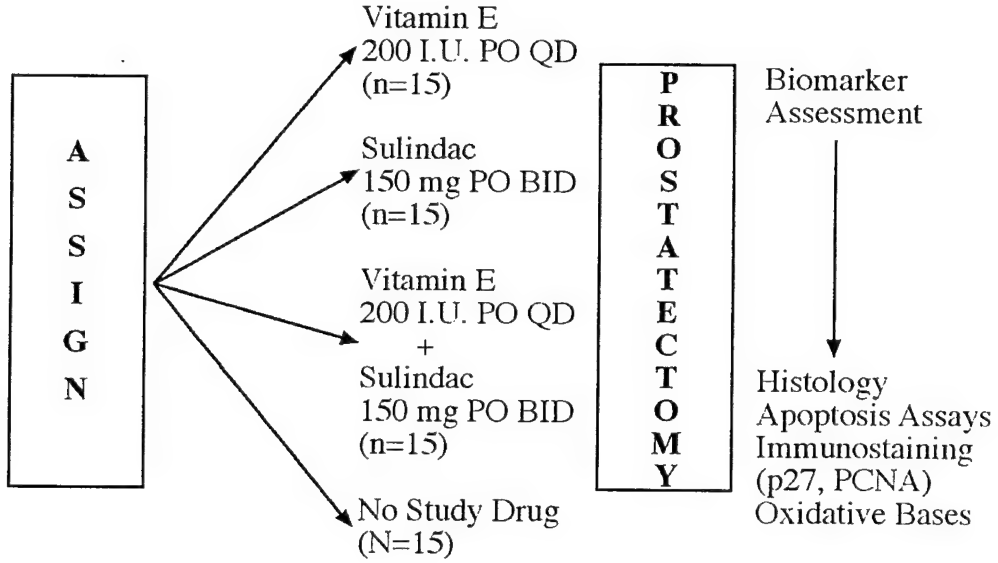
Schema

N=45 treatment patients + 15 controls

Second Opinion (Biopsy Review)
New Patient (Diagnostic Biopsy)
8 (end Study)



Adenocarcinoma of the Prostate
One or More of the Following Required
Gleason sum ≥ 7
Pre-study PSA ≥ 15 ng/dl
Stage T2b or T2c
≥ 45% risk of capsular penetration based on Partin Tables



STUDY RATIONALE

One in five men in the US will develop invasive prostate cancer. The development of potent prevention strategies to diminish or eliminate this threat are in order and is the long term objective of this project. Progress in this field is upon a critical threshold as the basic biology of prostate cancer development and progression advances and new agents are developed which may impact on cancer initiation. The daunting task and enormous costs associated with prevention trials could certainly be made easier if the "read out" of efficacy was shortened. Intermediate endpoints are controversial substitutes for the final results. Most intermediate endpoints at this time remain investigational just as the agents used in prevention trials are often investigational. Admittedly, the trial outlined in this proposal is critical and essential to the progress of prevention strategies in prostate cancer.

The progression of adenocarcinoma of the prostate within the prostate occurs through multi-step carcinogenesis, and has been compared to colorectal tumorigenesis, although the exact sequence of events is not yet known. The progression through clear pathologic steps including prostatic intraepithelial neoplasia is of unclear significance. It has been shown that prostate cancer tissue contains increased levels of prostaglandins and cyclooxygenase-2 (COX-2) compared with normal tissue.(8). Nonsteroidal anti-inflammatory agents (NSAIDs), which are nonselective cyclooxygenase inhibitors, have been shown to reduce the elevated levels of prostaglandins and COX-2 in tumor tissues such as in colon. Therefore, we believe that NSAIDs may halt the progression of adenocarcinoma of the prostate as well.

Vitamin E is a classic antioxidant. It has been shown that Vitamin E may be protective against certain epithelial cancers. A trial by Heinonen, et al, reported that consumption of 50 mg/day of Vitamin E was associated with a 40% reduction in the appearance of clinically-evident prostate cancer. Cyclooxygenase-2 (COX-2) inhibitors were developed to treat inflammatory diseases such as rheumatoid arthritis and osteoarthritis. Extensive preclinical and clinical data have been obtained that show potent anti-inflammatory activity. **We hypothesize that the administration of Vitamin E or Sulindac over a 6-8 week time period will result in drug-specific biomarker modulation in normal and cancer-containing prostate tissue obtained at prostatectomy. This biomarker modulation may provide the impetus for a large scale trial of Vitamin E or COX-2 inhibitors as preventive agents in men at risk for prostate cancer.** We propose to perform a pilot, single institution chemoprevention trial. Vitamin E or Sulindac will be administered over a 6-8 week in patients with clinically localized adenocarcinoma of the prostate prior to prostatectomy. The selected panel of biomarkers will be used to determine the bioactivity of this agent in prostate cancer.

1. OBJECTIVES

1.1 To determine whether the 6-8 week administration of Vitamin E 200 I.U. PO QD, Sulindac 150 mg PO BID, or Vitamin E 200 I.U. PO QD and Sulindac 150 mg PO BID, in this patient population will result in identifiable histologic changes, changes in serum markers (PSA, free/total PSA, etc), and markers indicative of apoptosis and growth arrest.

1.2 To characterize and describe the level of oxidized DNA bases in prostate tissue in treated patients and compare with oxidized base levels from untreated patients.

1.3 To store collected tissue and blood samples that may be evaluated with developing assays.

The successful completion of these objectives will provide information regarding the potential role of Vitamin E and cyclooxygenase inhibitors in the prevention of prostate cancer. Biologic generalizations of this will permit inferences regarding the effect of these agents in other cancers. These will provide a basis for the design and conduct of definitive prevention trials (i.e., large studies with definitive clinical outcomes, such as the occurrence of new malignancies). Additionally, these trials may provide information regarding the types of patients who are likely to benefit from Vitamin E or cyclooxygenase inhibitor therapy.

2. BACKGROUND AND PHARMACEUTICAL INFORMATION

2.1 Vitamin E and Sulindac Data

2.1.1 The precise role of Vitamin E in humans is not known. It is a classic antioxidant and can protect polyunsaturated fats from oxidative injury. Vitamin E insufficiency in humans can result in hemolytic anemia in neonates and muscle weakness in both adults and children. Sulindac is a non-steroidal anti-inflammatory drug. Its anti-inflammatory action seems related to inhibition of prostaglandin synthesis during inflammation. A sulfide metabolite of Sulindac seems to possess the biologic activity of this drug. The metabolites are eliminated in the feces and some are present in urine.

2.1.2 Clinical Pharmacokinetics

Absorption of Vitamin E occurs in the gut where 20-60% of available Vitamin E is absorbed. Plasma levels of Vitamin E vary with the normal range between 6-14μg/ml but levels do not correlate with Vitamin E total stores. Vitamin E is excreted in the bile after undergoing liver metabolism. Sulindac is about 90% bioavailable but the extent of absorption decreases with food. Peak plasma concentrations occur about 2 hours after administration under fasting conditions. With food intake, the absorption is about 3-4 hours. The mean half-life of Sulindac is 7.8 hours and its sulfide metabolite has a half-life of 16.4 hours. Sulindac and the sulfide metabolites undergo glucoronidation and about 50% is excreted in the urine in 4 days and 25% in the feces in 4 days.

2.1.3 Clinical Data

The recommended daily allowance of Vitamin E for adult men is 15 units per day. Vitamin E is prescribed in patients that suffer from fat malabsorption to prevent

Vitamin E deficiency. Sulindac is generally prescribed for the treatment of arthritis at a starting dose of 150 mg BID with doses generally not exceeding 400 mg daily. The dose of Sulindac used in the chemoprevention trials for colorectal polyps were 150 mg BID. There were no adverse side effects secondary to Sulindac in this trial and compliance was 85%.

2.1.4 Side Effects

a. Gastrointestinal

Large doses of Vitamin E (> 300 units per day) can cause nausea, diarrhea and intestinal cramps. Gastric ulceration and bleeding have occurred in patients treated with Sulindac. Some 10% of patients complain of GI pain with nausea reported in about 3-9% of patients. The rate of GI side effects in patients taking 200-400 mg of Sulindac daily is similar to patients receiving 600-1200 mg of ibuprofen daily.

b. Platelet

Sulindac has no effect on collagen-induced whole blood platelet aggregation or prothrombin time. Sulindac may prolong template bleeding times in 8-25% of patients treated with 400 mg of Sulindac daily for 7 days.

c. Drug Interactions

Vitamin E may increase absorption of Vitamin A. Vitamin E or its metabolites have been reported to have anti-Vitamin K effects which may result in an increased risk of bleeding in patients on oral anticoagulants. High doses of Sulindac could theoretically displace other protein bound drugs like oral anticoagulants or antidiabetic agents with adverse effects including enhancing the hypoprothrombinemic effects of coumadin. DMSO, Probenecid and Methotrexate should not be administered together as a result of alteration of plasma concentrations of these agents by Sulindac.

d.. Renal Toxicity

Renal impairment, interstial nephritis and nephrosis have been reported in patients treated with Sulindac. Several reports suggest that Sulindac is less likely to inhibit renal prostaglandins and thus result in less renal dysfunction than other available NSAID's.

2.1.5 Formulation

Vitamin E is supplied as a capsule and is stable in air and light. Sulindac is

supplied as a tablet. It is practically insoluble in water at pH < 4.5, and is slightly soluble in alcohol.

2.1.6 Storage Temperature

Storage of the drugs will be in the pharmacy, kept at room temperature and in a secured room.

2.2 Rationale for Prostate Cancer Prevention Study with Vitamin E and Sulindac

2.2.1 Epidemiology of Prostate Cancer in the US

Prostate cancer is the most common cancer diagnosed in men in the U.S. The American Cancer Society estimates of 1998 incidence approximate 184,000 new cases, with nearly 41,000 deaths. One in five men in the US will develop invasive prostate cancer. Men with a family history and African-American men are at the greatest risk of developing clinically significant prostate cancer. A recent autopsy study from Detroit found that 30% of men in their 30's had pathologic evidence of prostate cancer. Prostate cancer is a significant financial and emotional burden to our society.

Treatment options for localized cancer are expanding, but are primarily surgical removal (prostatectomy) or irradiation. Treatment for progressing or recurrent cancers include androgen ablation, often followed by palliative care with radiation and chemotherapy strategies. The focus on developing prevention strategies is critical to reducing the burden of this disease on society and to improving the lives of millions of men threatened with the prospect of prostate cancer.

2.2.2. Prostate Cancer Progression Model

The model of histologic progression of prostate adenocarcinoma has been outlined by Isaacs, et al(13,14). The sequence of chromosomal alterations and genetic changes is not as well established as the colon tumor progression model, yet many genetic changes have been identified including the most common genomic change that occurs in prostate cancer (methylation of the CpG islands and down-regulation of glutathione- S- transferase pi (GST -π) gene (15). Many studies are attempting to discover and place the genetic changes and other abnormalities in the progression model. Most recently, DeMarzo et al proposed the earliest lesion in prostate cancer may be an inflammatory regenerative atrophy (IRA), which allows replication in a highly oxidative environment that could damage DNA and lead to accumulated damage in cellular areas that are not protected. DeWeese et al has demonstrated that the accumulation of oxidative bases are promutagenic and that in prostate cancer where

GST-π is down-regulated or absent, NSAIDS may be important agents to halt this oxidative stress. Hence, our interest in NSAIDs and chemoprevention.

2.2.3 Vitamin E, NSAIDS and Chemoprevention

There are a number of pharmacologic agents which specifically target enzymes known to be involved in the production of reactive oxygen species. There is recent data suggesting several of these agents may be protective against prostate and other epithelial cancers. The trial by Heinonen, et al, reported that consumption of 50 mg/day of vitamin E was associated with a 40% reduction in the appearance of clinically-evident PCa (13). Intriguing data also exist for the drug sulindac. Sulindac treatment of patients with familial adenomatous polyposis (FAP), attributable to *APC* gene mutations, results in a reduction of colon polyp number and size (14). Mice with polyposis attributable to disrupted *Apc* genes exhibit attenuated colorectal polyp formation when treated with a specific COX-2 inhibitor (15). Sulindac has also been shown to inhibit the development of lung and breast tumors in animals (16, 17). Finally, there is *in vitro* data suggesting that human PCa cell line growth is also inhibited by sulindac treatment (18).

Three major types of evidence support the use of NSAIDs in chemoprevention: epidemiologic data, animal studies and human clinical trials. Many epidemiologic studies of aspirin and NSAIDs and colorectal cancers have been reported (17). Most of the studies showed a decreased risk of colorectal polyps or cancer with the use of aspirin or NSAIDs. Several epidemiologic studies have correlated aspirin use with a reduction in esophageal cancer incidence: Thun et al. showed that aspirin use was inversely associated with the development of fatal esophageal cancers (18). Animal studies of colorectal cancer provide support for the chemopreventive effects of NSAIDs. Among many experiments that have been performed on murine models induced with various carcinogens, Narisawa et al. have shown that indomethacin inhibits the development of colon tumors by methylnitrosourea in rats (19).

Therefore, in this chemoprevention trial, we propose to conduct the first evaluation of the effect of Vitamin E, an antioxidant and Sulindac, a non-selective COX-2 inhibitor. The endpoints will evaluate the effects of these agents on histologic, apoptotic, oxidative in normal and prostate cancer tissue.

Vitamin E is a classic anti-oxidant and is widely consumed by the public for its presumed anti-cancer and anti-aging properties. Sulindac is an FDA-approved non-selective cyclooxygenase (COX-2) inhibitor. Cyclooxygenase-2 is an inducible enzyme, whereas cyclooxygenase-1 is a constitutive enzyme. COX-2 is upregulated in inflammatory situations such as rheumatoid arthritis. In clinical trials, Sulindac has shown clinical benefit in reduction of analgesic use in rheumatoid arthritis and osteoarthritis patients. There is no significant alteration

of collagen-induced whole blood platelet aggregation following Sulindac treatment. Template bleeding time was > 15 minutes in 8-25% of patients treated with 200 mg PO BID for 7 days.

2.2.4 Possible Mechanisms of Chemoprevention with Vitamin E and NSAIDS

Vitamin E is known to be a scavenger of reactive oxygen species. These reactive oxygen species can inflict damage on DNA, some of which can be promutagenic (e.g. 8-hydroxydeoxyguanosine). Diminishing exposure of DNA to high levels of reactive oxygen species is thought to be protective. There are many possible mechanisms whereby NSAIDs effect chemoprevention. In reviewing the arachidonic acid pathway, the enzyme cyclooxygenase itself may have a role in cancer progression. Earnest, et al., have studied the possibility that cyclooxygenase enzyme may activate procarcinogens in food products to carcinogens (26). In addition to formation of the prostaglandins, one of the end products is malondialdehyde (MDA), a carcinogen in rats (27). Prostaglandins also have been shown to affect cell proliferation and PGE2 has been shown to increase tumor growth (28).

As a group, NSAIDs are drugs with antiproliferative effects. Bedi et al. have shown that NSAIDs increase the rate of apoptosis in colorectal cells (29). NSAIDs also block cell replication at G0/G1 phase (30). Kawano et al. have postulated that NSAIDs affect neutrophils by making them adhere to each other and bind to blood vessel endothelin. This results in a decreased blood supply to the area and leads to damage to the mucosa (31). Brooks et al. have shown that NSAIDs interact with phospholipase C, and therefore, arachidonic acid bound to the cell membrane is not readily freed to start the entire metabolism pathway (32).

2.2.5 Intermediate Biomarker Endpoints

The challenge in chemoprevention trials is to determine intermediate endpoints. The surrogate endpoint biomarkers commonly addressed are in three categories: proliferation, differentiation, and genetic alteration/regulation (33). In the sulindac trial in FAP patients, size and numbers of colon polyps were measured. In cancer trials, tumor measurements are monitored by imaging studies and physical exam. However, in prostate cancer, the prostate is not a routinely accessible organ, like the esophagus or colon. Assessment of the response to radiation therapy for localized disease remains a difficult task because of the inability to measure and to follow change in the end organ. PSA is only partially helpful in this setting and its inadequacies beg for improved intermediate biomarker endpoints. In this chemoprevention trial, we propose to use various intermediate biomarker endpoints to determine efficacy of Vitamin E and Sulindac in prostate cancer patients

We will measure both molecular apoptotic markers and proliferation markers in this study, and correlate the results with observed histologic changes. There are valid reasons to study apoptotic markers. NSAIDs may increase apoptosis, and apoptotic markers have already been studied in prostate cancer tissue(38). We will measure the following markers: $p27^{Kip1}$ and PCNA. These markers will examine the effect of Vitamin E and COX-2 inhibition on cell cycle dynamics and proliferation.

The cyclins and their kinases play a role in cell cycle control. Regulators of the cell cycle likely play an important role in the response of prostate cells to a chronic oxidative stress. One such cell cycle regulatory protein thought to be important in this response to stress is $p27^{Kip1}$ (35). This is a cyclin-dependent kinase inhibitor that acts as a barrier to cell progression into the S-phase of the cell cycle by arresting cells in G_1 or a G_0-like state. Importantly, *P53* function has previously been shown to be less important in the response of human PCa to oxidant stresses like radiation (36), and therefore the role of $p27^{Kip1}$ in the DNA damage response of the cell is likely critical. Recently, several authors have reported that both PIN and PCa exhibit a decreased expression of $p27^{Kip1}$ (37, 38), thereby rendering the cells capable of entry into S-phase despite sustaining DNA damage. The resultant cell is one which exhibits down-regulation of $p27^{Kip1}$ polypeptide levels, required to prevent replication and segregation of a damaged DNA template, and is already vulnerable to accumulation of oxidized DNA bases because of inactivated *GSTP1* alleles. The combination of these two events would seem to be particularly deleterious and would heighten the risk of neoplastic transformation. It is possible that prostate cells which express both GSTP1 and $p27^{Kip1}$ polypeptides would accumulate low levels of oxidized DNA bases because the cells express both a protective enzyme and a protein capable of inducing cell cycle arrest following DNA damage in order to repair it and/or prevent further accumulation. Cells which express GSTP1 but not $p27^{Kip1}$ polypeptide or cells which express neither may be more likely to accumulate higher levels of oxidized DNA adducts.

Histologic markers of proliferation may be useful in determining the degree to which the cell's DNA has been damaged by oxidant stress or inhibited by treatment with Vitamin E or Sulindac. PCNA should not be present in quiescent or growth arrested cells (33). Therefore, one might expect that cells that accumulate oxidized DNA bases, and are tolerant to that type of damage or have minimal defense against it (e.g. inactivated *GSTP1* alleles), would continue to proliferate and express PCNA. On the other hand, cells which accumulate lower levels of oxidized bases because they are less tolerant of this accumulation and/or have defense mechanisms in place to prevent large accumulations of oxidized bases (e.g. intact GSTP1 protein function) and stop proliferating, would not express PCNA. Vitamin E and Sulindac would be expected to prevent

cellular accumulation of oxidized bases, therefore treated patients are hypothesized to have a decrease in PCNA expression.

Apoptotic cells will be identified histologically and by the method of terminal deoxynucleotidyl transferase (TdT)- mediated deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL).

The amount of oxidized DNA adducts, such as 8-OHdG, present in the prostate of patients with PCa has not been established. Importantly though, it has been previously shown that one can measure a number of these adducts in DNA from tissue and that these measurements, in certain series, correlate with cancer risk (10, 11, 12). It is also known that diets high in fruits and vegetables and low in saturated fats can diminish the risk of certain forms of cancer (5). The precise reason(s) that this type of dietary strategy successfully diminishes cancer risk is not clear, but it may be related to the consumption of foods rich in anti-oxidant micronutrients (5). Sulindac is potentially one pharmacologic agent that may specifically target enzymes known to be involved in the production of reactive oxygen species. There is recent data suggesting several of these agents may be protective against prostate and other epithelial cancers. As previously stated, intriguing data exists for the drug sulindac. Sulindac treatment of patients with familial adenomatous polyposis (FAP), attributable to *APC* gene mutations, results in a reduction of colon polyp number and size (14). Mice with polyposis attributable to disrupted *Apc* genes exhibit attenuated colorectal polyp formation when treated with a specific COX-2 inhibitor (15). Sulindac has also been shown to inhibit the development of lung and breast tumors in animals (16, 17). Finally, there is *in vitro* data suggesting that human prostate cancer cell line growth is also inhibited by sulindac treatment (18). There is conflicting data on whether consumption of any of these agents can produce a decrement in the type and number of promutagenic DNA adducts (19, 20). We hypothesize that patients with prostate cancer will exhibit a large amount of oxidized DNA adducts as a result of *GSTP1* gene inactivation and the chronic oxidant stresses to which they are exposed. We also hypothesize that the number of DNA adducts can be diminished by treatment of patients with pharmacologic agents such as Vitamin E and Sulindac. These data provide the rationale for a clinical investigation of cellular oxidative damage in patients with prostate cancer. Previous work has revealed that accumulation of high levels of the oxidized base 8-OHdG can be promutagenic in some systems (4). Ames, et al have proposed that high levels of oxidized DNA adducts reflect exposure to a chronic oxidant stress and that one can accurately measure and follow these levels in the DNA of humans. A number of methods have been used to determine these levels, including HPLC-ECD, GC/MS-SIM and HPLC-MS. Each method has its strengths and weaknesses and we have experience using both HPLC-ECD and GC/MS-SIM (24).

2.3 Rationale for the Pre-surgical Window for Treatment with Vitamin E and Sulindac

Prevention strategies seek reduction of cancer development in high risk patient populations. Prostate cancer is often a multi-focal lesion within the prostate, including areas of prostatic intraepithelial neoplasia (PIN) to frank carcinoma. The reversion or regression of PIN is theoretically possible, but due to sampling errors changes in PIN cannot be accurately and reliably evaluated. Intermediate endpoints of drug bioactivity as documented by changes in treated prostate cancer tissue, normal an malignant, is an appropriate first step before embarking on large scale prevention trials. The patients that will undergo treatment on this clinical study will have frank carcinoma. Changes in a panel of biomarker variables consistent with drug activity will be obtained and compared to similar data collected in untreated patients. This is not a prevention trial. It is a trial to explore the feasibility of Vitamin E and Sulindac as a preventive agent as a modulator of molecular pathways employed by cancer cells during progression.

As many of the biomarker assays are dependent on adequate tissue procurement and tissue sample volume, patients with a significant probability of capsular penetration or prostate-confined bulky disease will be identified by using standard predictive criterion (The Partin Tables, Appendix 18.9). Over 850 men undergo radical prostatectomy at the Johns Hospital each year. Approximately 300 patients will be eligible for this study each year based on the criterion outlined: >45% risk of capsular penetration – various combinations of Gleason sum ≥ 7, Pre-study PSA ≥ 15 ng/dl, or Stage T2B, T2C). Patients will be approached by the treating urologic surgeon about participating in the trial if the decision for radical prostatectomy has been made. Patient enrollment and prostatectomies will be performed at the Johns Hopkins Hospital. It is expected that patient enrollment will average 5 patients/month. All patients will undergo follow-up at this institution.

3. PATIENT SELECTION

3.1 Patient Sample

3.1.1 Sample size: A total of 60 patients will be enrolled on this clinical trial. Patients will be consecutively assigned to treatment with Vitamin E, with Sulindac, with Vitamin E and Sulindac or with no study drugs. Up to 10 additional patients will be enrolled to replace patients not completing their treatment assignment.

3.1.2 Gender: Male patients with localized prostate cancer

3.1.3 Age: Patients must be at least 18 years of age

3.1.4 Race: Minorities will be actively recruited. No exclusion to this study based on race.

3.2 Eligibility Criteria. (Patients must meet all criteria)

3.2.1 Patients must have a histologically or cytologically documented diagnosis of prostate cancer meeting one or more of the following criteria:

a. Gleason sum ≥ 7
b. Pre-study PSA ≥ 15 ng/dl
c. Clinical stage T2b, T2c
d. Any combination of PSA, Stage, Gleason sum with an estimated > 45% chance of capsular penetration.

3.2.2 The patient has decided upon radical prostatectomy at the Johns Hopkins Hospital as treatment choice for localized prostate cancer.

3.2.3 Patients must have a performance status of 0 or 1 (Eastern Cooperative Oncology Group Performance Status - See appendix 17.1)

3.2.4 Patients must have had no recent major surgery, radiotherapy, hormonal, or chemotherapy in the past 28 days and be fully recovered from toxicity.

3.2.5 Patients must have adequate bone marrow function at study entry. (WBC > 3000 or ANC > 1500/mm^3, platelets >100,000/mm^3, and hemoglobin > 9g/dl)

3.2.6 Patients must have satisfactory renal and hepatic function, defined as plasma creatinine of ≤ 1.5 mg/dl, total bilirubin < 1.5, and AST/ALT < 1.5 x the upper limit of normal.

3.2.7 Patients must have no allergy to medications containing sulfa.

3.2.8 No active infectious process may be present, including HIV or viral hepatitis.

3.2.9 Patients must have no medical or psychiatric problems unrelated to the malignancy of sufficient severity to limit full compliance with the study or expose them to undue risk.

3.2.10 Patients must be able to provide informed consent and to return to the clinic for adequate follow-up as required by the protocol.

3.2.11 Patients with ≥ 3 cores positive unilaterally are eligible.

3.3 Exclusion Criteria

3.3.1 Chronic use of Vitamin E or NSAIDs/glucocorticoid use 4 weeks prior to entry (chronic use defined as frequency more than 3 times per week for more than 2 weeks).

3.3.2 Patients initiating supplements of vitamins (except multivitamin) or herbs with known effects on prostate function, including PSA within one month prior to study entry are excluded.

3.3.3 Necessary use or maintenance or concurrent Vitamin E or NSAIDs/glucocorticoids during trial.

3.3.4 History of bleeding disorders

3.3.5 History of chronic use of anticoagulants

3.3.6 History of chemotherapy, radiation therapy, or surgery within 4 weeks prior to study entry.

3.3.7 History of androgen ablation, immunologic or investigational therapy for prostate cancer.

3.3.8 Patients initiating supplemental vitamins (excluding multivitamin) or herbs within 4 weeks prior to study entry

3.3.9 Confirmed evidence of metastatic disease secondary to prostate cancer.

3.3.10 Serum creatinine >1.5 mg/dl or creatinine clearance < 50 ml/min.

3.3.11 Patients with prior known Sulindac or other COX-2 inhibitor therapy are excluded from this study.

3.3.12 History of other active malignancy within past 5 years except for superficial bladder cancer and non-melanoma skin cancer.

4. REGISTRATION OF PATIENTS

All eligible patients for this study should be discussed with a principal investigator or the project manager before entry on-study. Patients will be identified by the urologists participating in the study. The research nurse or the physician will determine eligibility and patient interest in participating. A twice monthly meeting of all investigators will review all patients that may be eligible of study. Once a patient has consented, an on-study form should be completed, the on-study requirements for laboratory work and evaluation fulfilled (section 7.1), and informed consent obtained (Appendix 17.3). Randomization to cohort will occur independent of the investigators.

The Johns Hopkins Oncology Center and the Brady Urological Institute will be the only participating center. Dr. DeWeese (410-955-6980) is the principal investigator. Arrangements for treating patients on this protocol must be made by contacting the Research Nurse or the principal investigator.

Patients will be registered through the JHOC Clinical Research Office (Room 1105, 550 Building). Registration must be handled by the investigators and/or responsible research nurses.

5. TREATMENT PLAN

5.1 Treatment Plan Summary

All patients with newly diagnosed localized prostate cancer seen by the surgeons of Brady Urological Institute will be considered for eligibility for this trial. Many patients will have a biopsy specimen from an outside institution and will have their biopsy re-reviewed by our Pathology Department. A smaller percentage of men will have their diagnosis of prostate cancer made in the Urology clinic through standard biopsy procedures. These specimens are reviewed automatically by our Pathology Department. Those patients meeting eligibility criteria, including the patient decision to proceed with radical prostatectomy as their choice for local control, will meet with the research nurse and an investigator to review the study. **No patient will have their surgery delayed to meet the 6-8 week treatment schedule prior to prostatectomy.** It is quite common for patients to wait 6-8 weeks for their surgical procedure because of the practice of obtaining autologous blood in preparation for the procedure and because of operating room scheduling priorities.

Eligible patients will be consented and have their date of surgery assigned prior to initiating study drug. Patients will be assigned treatment with Vitamin E 200 I.U. PO QD, Sulindac 150 mg PO, Vitamin E 200 I.U. PO QD and Sulindac 150 mg PO BID, or no study drug for at least 6 weeks (42 days) duration but not greater than 8 weeks (60 days). Patients will take the assigned study drug up until the evening before the surgery. The study drug should not interfere with the surgeons' practice of obtaining autologous blood. Patients will not be prescribed the study drug after the surgical procedure is completed.

Patients will have an initial history and physical examination, standard laboratory and pre-operative testing at baseline. A thorough review of patient medications, including use of vitamins and herbal therapies, will be obtained at baseline and reviewed with each visit. It is recommended that patients have blood sampling every two weeks while on study to determine effects of Vitamin E and Sulindac on PSA and other serum markers. Patients that live a substantial distance from this institution may have their PSA's obtained at outside laboratories, but be excluded from serum banking on those days because of inability to return to Hopkins for those visits. Each patient will either meet with the research nurse or discuss by phone any toxicity noted while on study. All patients are typically scheduled for a pre-operative evaluation in the weeks before surgery. This will be considered a standard visit for each patient, although a floating day in terms of follow-up. Pre-surgery, within 24 hours of the surgery, toxicity screening, a history and physical examination, review of medications, as well as blood tests including CBC, electrolytes, LFTs, PT/PTT will be performed. Patients will be given a list of over the counter medications, such as Tums, that are acceptable for use while on this study and also given a list of NSAIDs and aspirin-containing products to avoid while in this study.

Plasma/serum/buffy coat will be stored at on this schedule as well. Patients will consent to allow their prostate gland be evaluated as per standard pathologic stage and grading and for evaluation in the research laboratory for modulation of molecular markers induced by treatment with these agents.

5.2 Treatment Assignment

When patients are enrolled in the study, they will be consecutively assigned to treatment with either Vitamin E 200 I.U. PO QD, Sulindac 150 mg PO BID, Vitamin E 200 I.U. PO QD and Sluindac 150 mg BID, or neither Vitamin E nor Sulindac (control group). Each patient will be assigned an identification number in sequence, and increasing by units of one.

5.3 Method of Administration

Vitamin E or Sulindac will be dispensed in 2-4 week supplies, depending on scheduled follow-up with a clinic visit. Patients will self-administer the pills. The patients should take the pills with meals or small snack. Diaries to record administration, concomitant medications including other vitamins and herbs, compliance, and toxicity will be completed by patients or their caregiver.

Of note, patients will be asked to refrain from adding other vitamin or herbal supplements during the pre-operative period.

5.4 Treatment Adjustments

5.4.1 Dose Limiting Toxicities

For the purposes of this study, dose limiting toxicities are defined as clinical and laboratory abnormalities that are related to therapy with study drugs, requiring drug discontinuation or significant dose reduction. These toxicities will be graded using the NCI Common Toxicity Criteria. (See Appendix 17.5) These are generally Grade 3 or Grade 4 toxicities.

DLTs may be detected from various sources, including patient complaints, clinical evaluations, physical signs and/or laboratory reports on blood chemistries, hematology, or urinalysis. It is very important to monitor closely all these parameters throughout the study for the patients' health and well being. Laboratory reports of toxicities should be communicated by telephone to the patient as soon as they are known, with appropriate instructions and a schedule to visit the clinic as soon as necessary, depending on the nature and severity of the laboratory abnormality.

5.4.2 Dose Adjustments for Toxicities

Dose adjustments may be made for a patient who develops a DLT or for a patient who cannot tolerate the therapeutic regimen to which he is assigned.

The following guidelines are to be used when a DLT occurs:

1. If the patient develops persistent upper gastrointestinal side effects, despite the addition of an agent which may induce acid suppression such as omeprazole, then Sulindac will be reduced by 25%.

2. Despite dose reduction, if the patient has grade 3 toxicity, then patient will have drug discontinued and be removed from study.

3. If clinically indicated, the patient may be taken off study at the discretion of the treating investigator.

4. If upper gastrointestinal ulcers (gastric or duodenal) develop, the patient will be taken off study.

5. Increase of creatinine > 1.5 or decrease of creatinine clearance of < 50, dose is reduced by 25%.

6. Should there be an adverse event such as significant bleeding that is uncontrolled and either transfusion or surgery is required, the patient will be taken off study.

A DLT related to treatment with Vitamin E or Sulindac may result in discontinuation and/or reduction in dose (see above). In those cases where a DLT occurs that is felt by the investigator to be not related or probably not related to therapy with Vitamin E or Sulindac, treatment with study medication may continue with appropriate monitoring according to standard medical practice.

5.5 Treatment Continuation

Patients will continue to take the agent assigned at study initiation up until the evening before the day of the planned surgical procedure. No patient will continue on study drug after their surgery. All remaining tablets will be returned with a formal pill count.

5.6 Termination of Treatment

Patients not complying with treatment assignment or those patients experiencing treatment-limiting toxicity may have their treatment terminated prior to their scheduled prostatectomy. Poor **compliance with therapy will not be a reason to forego surgery.** Patients may at any time decide to discontinue therapy and they should notify the investigator or research nurse of this decision. As always, the Investigator must consider the patient's best interest and document the specific reasons and rationale for the continued treatment or withdrawal if toxicity or patient personal issues arise. The occurrence of severe systematic toxicity or several instances of treatment-limiting toxicity may require that treatment be stopped and the patient be withdrawn from the study.

5.7 Incompatible Drugs

Acetaminophen is preferred over salicylates or non-steroidal anti-inflammatory agents for minor pain/headache relief.

6. ADVERSE EVENTS

The safety of all patients enrolled in this study will be monitored throughout the study. Safety monitoring will include history and physical examination with vital signs, adverse event reporting and laboratory evaluations. A follow-up safety evaluation will be done for each patient approximately four (4) weeks after discontinuing treatment / prostatectomy or withdrawal from the study for any reason. In addition, patients should notify the study staff of any problems that occur between visits by telephone and, if necessary, be evaluated by the Investigator or study staff at an unscheduled interim visit.

6.1 Adverse Event (AE) Definitions

The following definitions of terms guided by the International Conference on Harmonization and the US Code of Federal Regulations apply to this section:

An adverse event is an untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An adverse event is any condition that appears or worsens after initiating the use of study drug. All adverse events should be noted on the Adverse Reaction CRF, whether or not it is felt to be related to study drug.

Serious adverse event is any adverse event that is fatal or life-threatening, is permanently disabling, requires or prolongs inpatient hospitalization, or is a congenital anomaly, cancer, or over-dose.

Life-threatening, for the purpose of reporting adverse events, refers to an event in which the patient was, in the view of the initial reporter, at ***immediate*** risk of death at the time of the event as it occurred (i.e., it does not include a event that, had it occurred in a more serious form, might have caused death).

Associated with the use of the drug means that there is a ***reasonable possibility*** that the experience (event) may have been caused by the drug.

Unexpected adverse experience (event) means any adverse experience (event) that is not identified in nature, severity, or frequency in the current Investigational Drug Brochure.

6.2 Reporting of Adverse Events

All adverse events (AEs) occurring with any patient participating in this clinical trial must be reported to the Principal Investigator as described below.

Any serious adverse event, including any event resulting in death, which occurs during the study must be reported by telephone, within 24 hours of the investigatory learning of the event to:

Theodore L. DeWeese, M.D.
The Bunting- Blaustein Building
1650 Orleans St., Baltimore, MD 21231-1000
410-955-7068 or 410-614-3979

All adverse events, regardless of severity, and whether or not ascribed to the study drug administration, will be recorded in he appropriate section of the Case Report Form. Patients withdrawn from the study due to adverse events will be followed by the Investigator until the outcome is determined and, when appropriate, additional written reports and documentation will be provided.

6.3 Classification of Adverse Events by Severity

The Investigator must categorize the severity of each adverse event according to the following guidelines:

Criteria

NCI toxicity criteria will be followed. (Appendix 17.5). Special attention will be paid to gastrointestinal and renal toxicity.

Mild:
Grade I NCI Common Toxicity; or

if not found in the NCI Common Toxicity table, an adverse event that is asymptomatic or barely noticeable to the patient; not interfering with patient's daily activity performance or functioning; generally not requiring alteration or cessation of study drug administration; and/or ordinarily not needing therapeutic intervention.

Moderate:
Grade II NCI Common Toxicity; or

if not found in the NCI Common Toxicity table, an adverse event of sufficient severity as to possibly make the patient's daily activity performance of functioning; generally not impairing the patient's daily activity performance or functioning; generally not impairing the patient's ability to continue in the study; and/or possibly needing therapeutic intervention.

Moderately Severe:

Grade III NCI Common Toxicity; or

if not found in the NCI Common Toxicity Table, adverse event generally causing severe discomfort; significantly influencing the patient's daily activity performance or functioning; generally requiring alteration or cessation of study drug administration; and/or generally therapeutic intervention.

Severe:

Grade IV NCI Common Toxicity; or

if not found in the NCI Common Toxicity table, and adverse event that is considered to be life-threatening; resulting in significant disability or incapacity; and/or representing the worst possible occurrence of that event.

6.4 Classification of Adverse Events by Relationship to Study Drug Administration

The relationship of each adverse event to the study drug administration will be assessed by the Investigator after careful consideration, and according to the following guidelines:

NO, NOT RELATED

This category is applicable to those adverse events which are clearly due to extraneous causes (concurrent drugs, environment, etc.) and do not meet the criteria for drug relationship listed under PROBABLY NOT; POSSIBLY; PROBABLY; AND YES, RELATED.

PROBABLY NOT RELATED

This category applies to those adverse events which are judged to be unlikely to be related to the study drug administration. An adverse event may be considered to be PROBABLY NOT RELATED when it meets at least two (2) of the following criteria:

a) It does not follow a reasonable temporal sequence from administration of the study drug.

b) It could readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.

c) It does not follow a known or expected response pattern to the study drug.

d) It does not reappear or worsen when the study drug is readministered.

POSSIBLY RELATED

This category applies to those adverse events which are judged to be perhaps related to the study drug administration. An adverse event may be considered POSSIBLY RELATED when it meets at least one (1) of the following criteria:

a) It follows a reasonable temporal sequence from administration of the study drug.

b) It could not readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.

c) It follow a known or expected response pattern to the study drug.

PROBABLY RELATED

This category applies to hose adverse events which are felt with a high degree of certainty to be related to the study drug administration. An adverse event may be considered PROBABLY RELATED if it meets at least two (2) of the following criteria:

a) It follows a reasonable temporal sequence from administration of the study drug.

b) It could not be reasonably explained by the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.

c) It disappears or decreases on cessation or reduction in study drug dose. There are exceptions when an adverse event does not disappear upon discontinuation of the drug, yet drug relatedness clearly exists (e.g., bone marrow depression, fixed drug eruptions, tardive dyskinesia, etc.)

d) It follows a known or expected response pattern to he study drug.

YES, RELATED

This category applies to those adverse events which are incontrovertibly related to study drug administration. An adverse event may be assigned to this category if it meets at least the first three (3) of the following criteria:

a) It follows a reasonable temporal sequence from administration of the study drug.

b) It could not be reasonably explained by the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.

c) It disappears or decreases on cessation or reduction in study drug dose. There are exceptions when an adverse event does not disappear upon discontinuation of the drug, yet drug relatedness clearly exists (e.g., bone marrow depression, fixed drug eruptions, tardive dyskinesia, etc.).

d) It follows a known or expected response pattern to the study drug.

e) It reappears or worsens when the study drug is readministered.

6.5 Death on Study

If a patient expires while on study, permission for autopsy will be sought. This information, when available, will be used in correlation with the clinical data. Deaths on study must be reported to the Principle Investigator.

7. STUDY PARAMETERS

7.1 On- Study Assessments

Each patient must have the following evaluations prior to receiving the first dose or treatment assignment: A flow chart of study activities is in Appendix 18.6.

history and physical exam
vital signs
performance status assessment
determination of clinical stage
complete blood count with platelet and differential
chemistry panel (including electrolytes, creatinine, bilirubin,
AST, ALT, alkaline phosphatase, uric acid,
total protein, albumin, calcium,
phosphorous, magnesium)
PT, PTT
PSA, free/total PSA
pathologic confirmation of cancer diagnosis
signed informed consent document received in the JHOC pharmacy

7.2 Baseline Visit

All baseline procedures will be completed within 2 weeks before the first dose of study medication, unless otherwise noted.

At the baseline visit, the Investigator and/or study staff will:

- Review the study procedures and determine that eligible patient is willing to comply with protocol requirements.
- Review the inclusion and exclusion criteria with the patient and determine if the patient can be a participant in the study.
- Record previous and concomitant medications, including vitamin and herbal supplements for the 4 weeks before the first dose of study medication.
- Record a complete patient medical history.
- Perform a surgical eligibilty screening examination; record vital signs, height, and weight
- Document ECOG status.

- Collect blood and complete laboratory safety evaluations (CBC with platelet count, PT, APTT, Chemistry panel, Testosterone, PSA, free/total PSA
- Complete the enrollment form and fax it to the Biostatistics Office of The Johns Hopkins Oncology Center for patient number and dose group assignment.

7.3. Study Day 1

Study Day 1 begins when the patient receives his first dose of treatment assignment. Subsequent visits should be scheduled from the date of the Study Day 1 Visit.

At the Study Day 1 Visit, the Investigator and/or study staff will:

- Administer study medication
- Record patient's weight and ECOG performance status.
- Update the record of concomitant medications.
- Review the study requirements. The patient will be provided instructions and the telephone number to call for problems or questions.
- Pharmacokinetic sampling will be obtained depending on agent assigned (See Section 8)

7.4. Study Visits During Treatment

Study visits will occur every other week, if feasible, until the time of surgery. In addition, patients may notify the study staff of any problems between visits and may be seen in the clinic at unscheduled visits as warranted.

Study evaluations during treatment will be completed according to the following schedule:

- PSA measurements. PSA will be determined every 2 weeks until the time of surgery (This lab study may be performed at an outside lab if the patient is unable to come to JHH for this visit)
- Physical Examination. A directed physical examination will be performed every other week, if feasible
- ECOG Status. ECOG performance status will be determined every other week.
- Hematology, blood Chemistry: These tests will be performed at the pre-operative evaluation routinely scheduled for patients undergoing surgery.
- PT, APTT: These tests will be performed before surgery.
- Administration of study medication and collection of unused tablets. Study medication will be dispensed and collected every other week until surgery.

7.5 End of Treatment Visit/One Month Post-op

Treatment with Vitamin E or Sulindac will be discontinued on the day prior to surgery. Patients will be evaluated by phone interview by the research nurse or one of the investigators within one month post-operatively to evaluate for any late sequelae of

treatment. A PSA will be obtained at 3 months post-operatively. The operative note will be maintained to determine if any untoward effects are noted at the time of surgery.

8. BIOASSAYS

8.1 PSA tumor marker

The effect of Vitamin E and Sulindac on PSA or free/total PSA ratios is not known. In this 6-8 week trial, serum for PSA and free/total PSA will be obtained at baseline and every two weeks up until surgery. It is not expected that there will be a significant change in PSA during the short course of this study, however, if PSA changes are noted, defintions of PSA response follow:

Complete PSA Response: PSA measurements within normal limits on at least 2 consecutive determinations performed no less than 4 weeks apart.

Partial PSA Response: Decrease of $\geq$ 50 % in PSA levels, but without normalization of the PSA, when compared to the baseline value. The 50% or greater decrease in PSA must be confirmed on at least 2 separate, consecutive determinations performed no less than 4 weeks apart.

Stable PSA Disease: PSA decreased or increased by $\geq$ 50 % when compared to the baseline value. Such stability in the PSA must be confirmed on at least 3 separate, consecutive occasions performed no less than 4 weeks apart.

8.2 Immunohistochemistry of $p27^{kip1}$ and PCNA:

We will measure both molecular apoptotic markers and proliferation markers in this study, and correlate the results with observed histologic changes. There are valid reasons to study apoptotic markers. NSAIDs may increase apoptosis, and apoptotic markers have already been studied in prostate cancer tissue. We will measure the following markers: $p27^{Kip1}$ and PCNA. These markers will examine the effect of Vitamin E and Sulindac-induced COX-2 inhibition on cell cycle dynamics and proliferation.

The cyclins and their kinases play a role in cell cycle control. Regulators of the cell cycle likely play an important role in the response of prostate cells to a chronic oxidative stress. One such cell cycle regulatory protein thought to be important in this response to stress is $p27^{Kip1}$ (35). This is a cyclin-dependent kinase inhibitor that acts as a barrier to cell progression into the S-phase of the cell cycle by arresting cells in G_1 or a G_0-like state. Importantly, *P53* function has previously been shown to be less important in the response of human PCa to oxidant stresses like radiation (36), and therefore the role of $p27^{Kip1}$ in the DNA damage response of the cell is likely critical. Recently, several authors have reported that both PIN and PCa exhibit a decreased expression of $p27^{Kip1}$ (37, 38), thereby rendering the cells capable of entry into S-phase despite sustaining DNA damage.

The resultant cell is one which exhibits down-regulation of $p27^{Kip1}$ polypeptide levels, required to prevent replication and segregation of a damaged DNA template, and is already vulnerable to accumulation of oxidized DNA bases because of inactivated *GSTP1* alleles. The combination of these two events would seem to be particularly deleterious and would heighten the risk of neoplastic transformation. It is possible that prostate cells which express both GSTP1 and $p27^{Kip1}$ polypeptides would accumulate low levels of oxidized DNA bases because the cells express both a protective enzyme and a protein capable of inducing cell cycle arrest following DNA damage in order to repair it and/or prevent further accumulation. Cells which express GSTP1 but not $p27^{Kip1}$ polypeptide or cells which express neither may be more likely to accumulate higher levels of oxidized DNA adducts.

Histologic markers of proliferation may be useful in determining the degree to which the cell's DNA has been damaged by oxidant stress or inhibited by treatment with Vitamin E or Sulindac. PCNA should not be present in quiescent or growth arrested cells (33). Therefore, one might expect that cells that accumulate oxidized DNA bases, and are tolerant to that type of damage or have minimal defense against it (e.g. inactivated *GSTP1* alleles), would continue to proliferate and express PCNA. On the other hand, cells which accumulate lower levels of oxidized bases because they are less tolerant of this accumulation and/or have defense mechanisms in place to prevent large accumulations of oxidized bases (e.g. intact GSTP1 protein function) and stop proliferating, would not express PCNA. Vitamin E and Sulindac would be expected to prevent cellular accumulation of oxidized bases, therefore treated patients are hypothesized to have a decrease in PCNA expression.

8.3 TUNEL Assay for Apoptosis:

Apoptotic cells will be identified histologically and by the method of terminal deoxynucleotidyl transferase (TdT)- mediated deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL).

8.4 Measurement of Oxidative Bases in DNA obtianed for Prostate Cancer Tissue

The amount of oxidized DNA adducts, such as 8-OHdG, present in the prostate of patients with prostate cancer has not been established. Importantly though, it has been previously shown that one can measure a number of these adducts in DNA from tissue and that these measurements, in certain series, correlate with cancer risk (10, 11, 12). It is also known that diets high in fruits and vegetables and low in saturated fats can diminish the risk of certain forms of cancer (5). The precise reason(s) that this type of dietary strategy successfully diminishes cancer risk is not clear, but it may be related to the consumption of foods rich in anti-oxidant micronutrients (5). Sulindac is potentially one pharmacologic agent that may specifically target enzymes known to be involved in the production of reactive oxygen species. There is recent data suggesting several of these agents may be

protective against prostate and other epithelial cancers. As previously stated, intriguing data exists for the drug sulindac. Sulindac treatment of patients with familial adenomatous polyposis (FAP), attributable to *APC* gene mutations, results in a reduction of colon polyp number and size (14). Mice with polyposis attributable to disrupted *Apc* genes exhibit attenuated colorectal polyp formation when treated with a specific COX-2 inhibitor (15). Sulindac has also been shown to inhibit the development of lung and breast tumors in animals (16, 17). Finally, there is *in vitro* data suggesting that human prostate cancer cell line growth is also inhibited by sulindac treatment (18). There is conflicting data on whether consumption of any of these agents can produce a decrement in the type and number of promutagenic DNA adducts (19, 20). We hypothesize that patients with prostate cancer will exhibit a large amount of oxidized DNA adducts as a result of *GSTP1* gene inactivation and the chronic oxidant stresses to which they are exposed. We also hypothesize that the number of DNA adducts can be diminished by treatment of patients with pharmacologic agents such as Vitamin E or Sulindac. These data provide the rationale for a clinical investigation of cellular oxidative damage in patients with prostate cancer. Previous work has revealed that accumulation of high levels of the oxidized base 8-OHdG can be promutagenic in some systems (4). Ames, et al have proposed that high levels of oxidized DNA adducts reflect exposure to a chronic oxidant stress and that one can accurately measure and follow these levels in the DNA of humans. A number of methods have been used to determine these levels, including HPLC-ECD, GC/MS-SIM and HPLC-MS. Each method has its strengths and weaknesses and we have experience using both HPLC-ECD and GC/MS-SIM (24).

8.5 Measurement of Patient Compliance

Traditional measures of patient compliance in clinical trials utilize pill counting. This trial will use pill counts to track compliance. The pharmacy will dispense a known quantitity of pills to each patient. Patients will be instructed to bring their pill bottles and remaining pills. The research nurse, or the pharmacist will keep a record of returned pills and correlate with number of pills dispensed and number of doses expected to have been taken over the time dispensed. Diaries may be useful for patients to record their self-administration schedule.

8.6 Serum/Tissue bank

Serum/ plasma specimens will be frozen and stored at –20 degrees C for possible study if other bioassays are developed which may be of interest in this patient population. Tissue samples will also be stored in standard fashion – snap frozen/formalin/embedded.

9. TUMOR RESPONSE CRITERIA

9.1 Primary Measures of Efficacy

No change in the primary tumor is expected during the short treatment exposure. All pathologic specimens will be handled in routine fashion by the operating room staff. A study technician will be available to the pathologist handling the specimen to assure that the tissue is handled appropriately for the intended bioassays.

1. Changes in the concentration of PSA in serum will be recorded, and used to correlate any molecular effects noted after laboratory evaluation of the primary tissue.

2. Assessment of index tumors, including Gleason grading, nodal involvement, pathologic staging will be conducted in usual fashion and will be provided to patient for prognostic information.

10. DATA ANALYSIS AND STATISTICAL INTERPRETATION

10.1 Efficacy Analysis

The Johns Hopkins Oncology Center Clinical Research Office and its Biostatistics Core will serve as the coordinating data center for this study. The objectives of the coordinating center are:

Provide the infrastructure necessary to support the conduct in monitoring of the clinical trialsSupervise the preparation, quality control and maintenance of a study databaseProvide timely and expert interim and final statistical analyses of data.

Provide administrative support for meetings and communications of the collaborations

Assist with the preparation of scientific reports

10.2 STUDY ANALYSIS

The primary efficacy variable will be the determined after pathologic review of all tumor specimens. The pathologist and technicians working with and interpreting bioassays will be blinded to patient treatment.

We anticipate that 15 patients per treatment arm will be sufficient to estimate the value and variability of each assay to be completed and described. For example, response can be assessed in terms of reduction in 8OHdG levels compared to levels in untreated patients. As an outcome variable, the change in the average 8OHdG level in each treatment arm can be compared.

We intend to estimate the average change in 8OHdG with a precision that is 50% of the person to person standard deviation. Precision will be defined in terms of the 95% confidence interval on the mean 8OHdG. Using this criterion, the width of the 95% CI will

be using N=45 treated patients, which will be comprised of patients with a range of risk for capsular penetration. Following the accrual of this sample size, or reaching this precision, individual parameters may require additional patients to be accrued to improve upon the precision of the comparison. Other similar outcome measures will include measures of apoptosis and proliferation. This will be summarized as group means and/or proportions as appropriate.

Linear tests for trend across dose groups will be performed using linear regression models. Without knowing the error variance, an exact power statement regarding the trend across doses cannot be made.

Secondary outcome measures include measurement of measurement of apoptosis by TUNEL assays, immunohistochemistry of $p27^{kip1}$ and PCNA and measurement of oxidized DNA bases. Tertiary outcomes include patient compliance data. These measures will be summarized on a descriptive basis only. No pre-planned hypothesis tests will contribute to the power considerations. Although such comparisons will be performed, they will not be represented as the primary outcome of the trial.

10.3 Safety Analysis

Safety data will include laboratory, history and physical, and adverse event reports on both the local and systemic signs or symptoms of the study patients. These data will undergo descriptive statistical analysis.

11. FORMS

On study as well as follow-up data will be recorded into the computerized Oncology Center Information System. Forms to be kept include the following:

11.1 Case Report Forms

These forms should be completed and updated by the principal investigators. Forms include eligibility data, history and physical, prior therapy data, concomitant medications, study drug administration, extent of disease, notes, course assessment, off study summary and flow sheets.

11.2 Follow-up Form

The follow-up form will be completed on all subsequent visits or evaluations.

11.3 Data Submission

Quarterly progress reports are due 14 calendar days after each reporting period, annual progress reports are due 14 days after each reporting period, and written adverse event

reports are due 10 calendar days after event. The final report is due at the contract expiration date. The results of this study will be presented at appropriate national scientific meetings and submitted for publication on a timely basis.

12. HUMAN SUBJECTS

Before activation, the protocol will have been reviewed and approved by the Clinical Research Committee of The Johns Hopkins Oncology Center, The Joint Committee on Clinical Investigations of The Johns Hopkins University.

All participants must sign an informed consent that will describe the objectives of the study and potential risks. All patient data reported on the case reports forms will be identified by the patient's initials and study code number only. Patients shall not be identified by name.

13. DRUG ACCOUNTABILITY AND DRUG ORDERING

Once the patient's eligibility is established and the individual has been registered, a supply of the drug may be ordered. Drug may be requested by completing a Clinical Drug Request and mailing it to the Oncology Center Pharmacy Drug Development Office. Excess study medication will be returned to the pharmacy.

14. APPROVAL OF THE STUDY

This study will become active when the final protocols have been approved by the appropriate institutional review boards and the DOD.

15. PROCEDURE FOR AMENDMENTS TO THE PROTOCOL

All revisions or amendments to the protocol must be approved by the appropriate institutional review boards.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1 FDA Form 1572: The Principal Investigator will sign an investigative statement (FDA Form 1572) prior to initiating this study stating that the study will be conducted in compliance with regulations for clinical investigations.

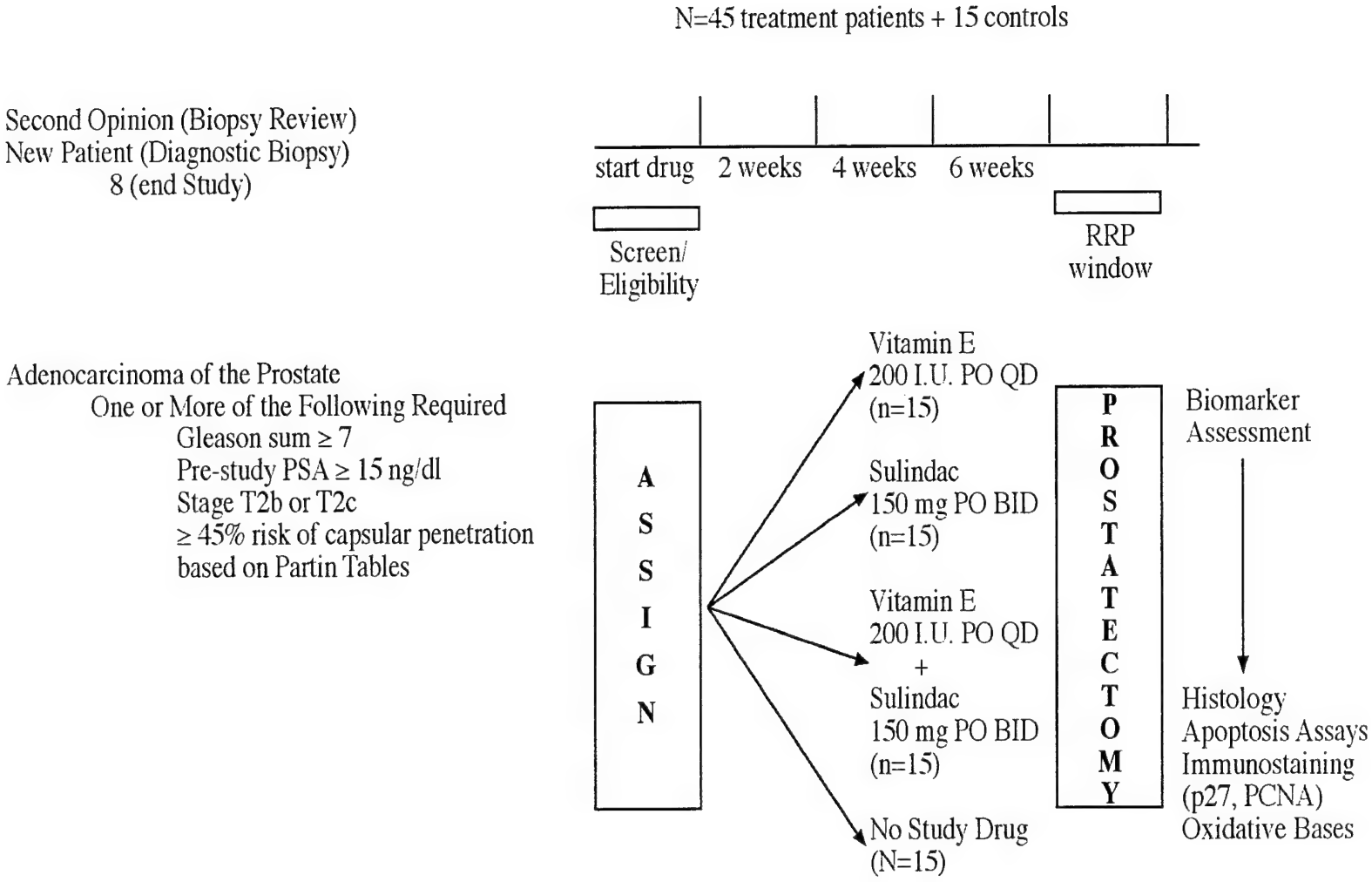
16.2 IRB: Prior to initiating the study, the Principal Investigator must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study protocol become necessary, protocol amendment will be submitted in writing to the IRB by the Principal Investigator for IRB approval prior to implementation.

16.3 Informed Consent: All potential candidates for the study will be given a copy to read of the Informed Consent for the study. The investigator will explain all aspects of the study in lay language and answer all the candidate's questions regarding the study. If the candidate

desires to participate in the study, he/she will be asked to sign the Informed Consent. Study drug will not be released to a subject without a signed Informed Consent. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice.

16.4 Record Retention: Clinical records for all subjects studied including history and physical findings, laboratory data, and results of consultations are to be maintained by the investigatory in a secure storage facility. These records are to be stored indefinitely.

Appendix - Schema



Appendix: EVALUATIONS DURING STUDY: SCHEDULE OF VISITS FOR STUDY PATIENTS/TIME OF TREATMENT (6-8 WEEKS)

PROCEDURE	VISIT I (ELIGIBILITY)	VISIT 2 (RANDOMIZATION- 0 MONTH, START DRUG)	VISIT 3,4,5 (Every 2 weeks, ON STUDY DRUG)	VISIT 6 (WITHIN 24 h of SURGERY)	VISIT 7
History	X			X	
Physical Exam	X			X	
CBC, Electrolytes, LFTS, PT/PTT	X		X	X	
PSA, Free/Total PSA	X	X	X	X	
Toxicity Monitoring			X	X	
Dispense meds		X	X		
Collect meds/pill count				X	
Histology (diagnostic)	X			X	
Histology (prostatectomy)				X	
Pre-op visit			X(floating)		
Biomarker Assessment TUNEL Immunohisto-chemistry Oxidative Bases				X	
Serum/Tissue Bank		X	X	X	

APPENDIX

APPENDIX

ELIGIBILITY CHECKLIST

1) Patients must have a histologically or cytologically documented diagnosis of prostate cancer meeting one or more of the following criteria:
   a. Gleason sum ≥ 7
   b. Pre-study PSA ≥ 15 ng/dl
   c. Clinical stage T2b, T2c
   d. Any combination of PSA, Stage, Gleason sum with an estimated > 45% chance of capsular penetration as determined by The Partin Tables

2) The patient has decided upon radical prostatectomy at the Johns Hopkins Hospital as treatment choice for localized prostate cancer.

3) Patients must have a performance status of 0 or 1 (Eastern Cooperative Oncology Group Performance Status - See appendix 17.1)

4) Patients must have had no recent major surgery, radiotherapy, hormonal, or chemotherapy in the past 28 days and must be fully recovered from toxicity.

5) Patients must have adequate bone marrow function at study entry. (WBC > 3000 or ANC > $1500/mm^3$, platelets >$100,000/mm^3$, and hemoglobin > 9g/dl)

6) Patients must have satisfactory renal and hepatic function, defined as plasma creatinine of ≤ 1.5 mg/dl, total bilirubin < 1.5, and AST/ALT < 1.5 x the upper limit of normal.

7) Patients must have no allergy to medications containing sulfa.

8) No active infectious process may be present, including HIV or viral hepatitis.

9) Patients must have no medical or psychiatric problems unrelated to the malignancy of sufficient severity to limit full compliance with the study or expose them to undue risk.

10) Patients must be able to provide informed consent and to return to the clinic for adequate follow-up appointments at the Johns Hopkins Oncology Center as required by the protocol.

11) Patients must be at least 18 years old

12) Patients with ≥ 3 cores positive unilaterally are eligible.

13) No concurrent or chronic use of Vitamin E or NSAIDs/glucocorticoid use 4 weeks prior to entry (chronic use defined as frequency more than 3 times per week for more than 2 weeks).

14) No supplemental use of vitamins (except multivitamin) or herbs within 4 weeks prior to study entry.

15) No necessary use, maintenance, or concurrent use of Vitamin E or NSAIDs/glucocorticoids during trial.

16) No history of bleeding disorders

17) No history of chronic use of anticoagulants

18) No history of chemotherapy, radiation therapy, or surgery within 4 weeks prior to study entry.

19) No history of androgen ablation, immunologic or investigational therapy for prostate cancer.

20) No confirmed evidence of metastatic disease secondary to prostate cancer.

21) Serum creatinine must be <1.5 mg/dl or creatinine clearance must be > 50 ml/min.

22) No prior known Sulindac or other COX-2 inhibitor therapy.

23) No history of other active malignancies within past 5 years except for superficial bladder cancer and non-melanoma skin cancer.

24) Adequate pre-operative condition.

Appendix 18.5 Proscribed Drugs

Drugs that interact with **sulindac and should not be taken within 24 hrs of starting treatment or while on the study**:

OVER-THE-COUNTER

- Aspirin
- Buffered aspirin
- BC powders/tablets, cold formulas
- Goodys powders/tablets, cold formulas
- Stanback powders/tablets, cold formulas
- Excedrin, Excedrin Extra, Excedrin IB, Saleto Tabs, Buffets II Tabs, Midol, Advil Cold and Sinus
- Alka Seltzer
- Ibuprofen (generic)
- Dristan Sinus, Ursinus Inlay tabs, Dimetapp Sinus, Valprin, Haltran Tabs, Doan's Pills, Magan
- Aspirin suppositories (generic-any strength)
- Naproxen (Aleve, Anaprox)
- Pepto-Bismol or products containing bismuth subsalicylate
- Products containing sodium salicylate and/or magnesium salicylates (Tricosal, Mobidin, Pabalate EC, Magsal)
- Ketoprofen
- Choline Salicylate
- Zantac (ranitidine)
- Tagamet (cimetidine
- Pepcid (famotidine)
- Axid (lansoprazole)

PRESCRIPTION MEDICATIONS

Brand name	**Generic**
• Celebrex	(celecoxib)
• Vioxx	(rofecoxib)
• Fiorinal, Fiorinal #3, Isollyl, Fortabs, Idenal, Axotal	(aspirin with codeine)
• Fiorgen PF, Lenorinal, Mornal, BAC	(aspirin + codeine + butalbital)
• Motrin, Rufen, Ibuprohn, Saleto, IBU	(ibuprofen)
• Azdone Tabs, Damason-P, Lortab, Panasol	(aspirin + hydrocodone)
• Percoden, Roxiprin	(aspirin + oxycodone)
• Synalgos	(aspirin + dihydrocodeine)
• Voltaren, Cataflam	(diclofenac)
• Naprosyn, Anaprox, Naprelan	(naproxen)
• Feldene	(piroxicam)
• Ansaid	(flurbiprofen)
• Orudis, Oruvail	(ketoprofen)
• Dolobid	(diflunisol)
• Clinoril	(sunlindac)
• Indocrin, Indochron ER	(indomethicin)

• Tolectin	(tolmetin)
• Daypro	(oxaprozin)
• Lodine	(etodolac)
• Meclomen	(meclofenamate)
• Nalfon	(fenoprofen)
• Ponstel	(mefenamic acid)
• Relafen	(nabumetone)
• Toradol	(ketoralac)
• Disalcid/Salsitab, Salflex, Argesic SA, Amigesic, Arthra-g, Mono-gesic	(salsalate)
• Trilisate	(choline/magnesium salicylate)
• Talwin Compound	(pentazocine + aspirin)
• Darvon Compound, PC-CAP	(propoxyphene + aspirin)
• Equigesic, Micrainin	(meprobamate + aspirin)
• Phenylbutazone	
• Rexolate, Tusal	(sodium Thiosalicylate)
• Prevacid	lansoprazole

Appendix 18.7: EVALUATIONS DURING STUDY: SCHEDULE OF VISITS FOR STUDY PATIENTS/TIME OF TREATMENT (>6 WEEKS)

PROCEDURE	VISIT I (Eligibility/on study)	VISIT 2,3,4, (Every 2 weeks, on study drug)	SURGICAL VISIT (WITHIN 24 h of SURGERY	PHONE INTERVIEW
History	X		X	X
Surgical Eligibility Screening Examination	X			
Physical Examination			X	
CBC, Electrolytes, SCr, LFTS, PT/PTT	X	X	X	
PSA, Free/Total PSA	X	X	X	X_1
Toxicity Monitoring		X	X	X
Dispense meds	X			
Collect meds/pill count		X	X	
Histology (diagnostic)	X		X	
Histology (prostatectomy)			X	
Pre-op visit		X(floating)		
Biomarker Assessment TUNEL Immunohisto-chemistry Oxidative Bases Micro-vessel density			(To be done in batches)	
Serum/Tissue Bank	X	X	X	

1 PSA only will be obtained at 3 months after surgery.

PURPOSE OF STUDY

You have prostate cancer that appears to be limited to your prostate gland. You have opted to undergo surgery to have your prostate gland removed. It is expected that your surgery will be scheduled in the next 6 - 8 weeks. In most cases, no other therapy for your cancer takes place other than the surgery. You are being asked to take part in a clinical trial where your doctors will look at your prostate gland and its cancer to see if any changes occur if you have taken a study drug in the weeks leading up to your surgery. The study drugs are in pill form and could be used in the future to prevent prostate cancer. We are looking for changes in your prostate cancer after treatment with one of these drugs to determine if a much larger study makes sense to answer the question of whether these drugs may be helpful. Your participation in this study is voluntary.

The study drugs include a vitamin, Vitamin E and a drug used in severe arthritis called Sulindac. Studies suggest that these drugs may slow the progression of some cancers down or even prevent them from forming. You have cancer and we do not know whether these drugs can produce these effects in humans. Usually no special drugs are given before surgery, so if you agree to participate, you may be assigned to treatment with one of the study drugs. If you are assigned one of the drugs you will take your assigned treatment for at least 6 weeks and up to 8 weeks prior to your surgery.

Nearly 60 patients will be enrolled in this study to take place at The Johns Hopkins Hospital. The study is sponsored by the United States Department of Defense.

PROCEDURES:

It will first be determined that you intend to undergo prostatectomy for the treatment of your prostate cancer. You will undergo a history and physical examination, and be asked to provide a list of all medications, including vitamins and herbs that you may take. Blood and urine samples will be obtained to assure your safety prior to starting the study drug.

You will be assigned Vitamin E, Sulindac, Vitamin E and Sulindac, or no study drug. Assignment will be in sequence. The first patient will receive Vitamin E, the second patient will receive Sulindac. The third patient will receive Vitamin E and Sulindac. The fourth patient will receive no study drug. The next four patients will receive the same sequence as the first four patients and all successive groups of four patients will continue in the same sequence until the study is complete. Both study drugs will come in pill form. If you are assigned Vitamin E, you will take the pills once per day. If you are assigned to Sulindac, you will take the pills twice a day. If you are assigned to both Vitamin E and Sulindac, you will take Vitamin E once a day and Sulindac twice a day. We ask you to save and return any pills that you do not use. If you take all the pills, we ask that you return the empty container. You will take the pills for at least six weeks before your surgery, but no more than 8 weeks. You will take the last dose of the medication on the night before your surgery.

You will be given a pill calendar and asked to mark off each day that you take the study drug. You will also be asked to list any side effects you many have to the drugs and to list any additional medications you are taking.

During the study you will be asked to return to the clinic to be evaluated for side effects and to follow-up on your blood tests. All other procedures are typical for a patient preparing for surgery at this institution. The blood tests may be done more often than is usually done in other patients similar to yourself. We will draw up to four tablespoons of blood with each sampling.

Your surgery will go on as planned. Your prostate gland will be removed and the pathologists will handle the tissue as they normally do. However, in your case, some portions of your tumor will be evaluated in research labs throughout the institution to examine if the study drug had any positive or negative effects. We will directly compare the results of those patients who received the study drug and those who did not.

You will not stay on the study drug after your surgery. You will be seen at your after surgery clinic visit to see if there were any other side effects noted by you.

Some of the tissue from your surgery and some blood samples will be stored for further research. Your identity will remain confidential on any blood or tissue sample that is used for research. The investigators may look at your records, but your name will be kept private. If any of the results are published, once again you will not be identified.

RISKS AND DISCOMFORTS:

With any drug, there may be side-effects. Vitamin E is generally very well-tolerated. Rarely, it can cause nausea, diarrhea and intestinal cramps in some patients.

Overall, Sulindac is well-tolerated. It is somewhat similar to drugs like aspirin or ibuprofen. Side effects may include irritation of the stomach lining, nausea, vomiting, an increase risk for bleeding, and a possibility that the drug may slow your kidney function down. There may be other side effects which are not known. You will be followed closely and be seen by one of the doctors or nurses working on the study every other week.

The blood tests may cause minor pain where the needle is placed to remove blood. Besides pain, there may be bleeding, soreness, and the possibility of infection. These risks are of the procedure, not because your blood is being drawn more often.

There is the possibility that you may have a slight increase in bleeding during your surgical procedure. Your surgeons are aware of this risk and will take standard caution during the procedure to limit blood loss.

You may stop treatment at any time if you have side-effects or no longer wish to participate. If you wish to stop treatment, please let your doctor or the research nurse know of your decision. Your decision in no way will interfere with your planned surgical procedure. You will continue to receive care form your surgeon.

Costs to your or your insurance company for participating in this study are those costs which are considered standard for someone with your condition. The study drugs will be provided free of charge. The research studies and assays on your prostate tissue will not be charged to you. You will be asked to

visit the research nurse during the six-eight weeks that you are on the study, and ;you will not be billed for those visits, unless it is your standard pre-operative visit to be scheduled by your doctor. As coverage by insurance carriers varies, you may wish to review your policy if questions regarding costs of clinical research are of concern.

If emergency treatment is needed as a result of this therapy or related to your cancer, you will be provided the best care possible at this institution. No money is available to reimburse you if emergency therapy is required.

BENEFITS:

There is no known benefit to participating in this study. Although these drugs may have some effects on your cancer cells, you may not receive it long enough to see improvement. We are looking for changes at a molecular level that will allow us to offer advice as to whether these drugs should be used more often. It is unlikely we will share with you the results of the molecular tests that we complete on your prostate tissue. Many of the tests are experimental and the meaning of their results is unclear at this time. We will not use any results to make decisions regarding your future care. If any data impacts on your care, you will be told of that finding.

ALTERNATIVES TO PARTICIPATION:

Alternatives to participation in this study include no treatment at all until your surgery or other investigational drugs. You will receive the best possible care at this institution no matter what your decision.

You should understand that this study involves research. Rules are made during the planning stages of this study and are used to make sure that patients who enter this study are suitable and fit for this therapy and that they have the defined medical problem to be treated. For your own well-being, as well as to ensure that the results of this study can be useful for making treatment decisions regarding other patients with similar disease, it is important that no exceptions be made to these rules for admission to the study. You will receive a copy of this consent form.